Chemical Library

MAR 10 1941

SCIENTIFIC EDITION

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

CONTENTS OF THIS ISSUE

The Toxicity and Trypanocidal Activity of Commercial Neoarsphenamine 33
C. A. Morrell and M. G. Allmark

Intravenous Toxicity of Heparin-Sodium Sulfapyridine Combinations and Protective Action of Barbiturates 38
N. A. David, N. M. Phalak, H. Donnell and H. Vehrs

Bacteriostatic Properties of Phenylacetic Acid . 41
C. F. Feasley and B. H. Gwynn, with Ed. F. Degering and P. A. Tetrault

The Synthesis of Several Dialkylmalonylguanidines with a Preliminary Note on Their Pharmacology 45
Orville H. Miller and Louis Fischer

Condensation of Amino Acids with Terpenes . 47
C. F. Krewson

The Qualitative Detection of Methanol 49
Walter C. Gakenheimer and Walter H. Hartung

Determination of Unsaturates in Cyclopropane 50
Frederick K. Bell and John C. Krantz, Jr.

Detection of Quinicine and Cinchonicine . . . 52
J. W. Millar and S. J. Dean

The Stabilizing Effects of Antioxidants upon Solution of Tannic Acid 53
K. P. DuBois and C. O. Lee

Homogeneity of Tablet Mixtures Before Granulating 56
E. C. Beeler and E. N. Gathercoal

A History of Blaud's Pills 60
M. L. Neuroth and C. O. Lee

Book Reviews 63

Abstracts 33–64

Advertising—Cover Pages 2, 3, 4 and pp. I to VIII

Published by the American Pharmaceutical Association. Publication office, 20th and Northampton Sts., Easton, Pa. Editorial office: Scientific Edition, 32 S. Greene St., Baltimore, Md. Practical Pharmacy Edition: 2215 Constitution Ave., Washington, D. C.

Annual Subscription: Journal of the American Pharmaceutical Association, complete $5.00; Canada, $6.00; Other Foreign, $6.50. (a) Scientific Edition, $4.00; Canada, $4.35; Other Foreign, $4.50; Single Numbers, $0.35; Canada, $0.40; Other Foreign, $0.50. (b) Practical Pharmacy Edition, $1.50; Canada, $1.80; Other Foreign, $2.00; Single Numbers, $0.15; Canada, $0.20; Other Foreign, $0.25.

No claims will be allowed for Journals lost in the mails due to: Failure to notify of change of address (thirty days' notice required); after a lapse of three months; failure to receive wrappers from returned Journals.

Entered as second-class matter January 23, 1917, at the Post Office at Easton, Pa., under the act of March 3, 1879, as 24 times a year: Scientific Edition monthly on the fifth. Practical Pharmacy Edition monthly on the 20th. Acceptance for mailing at a special rate of postage provided for in Section 1103, Act of October 3, 1917, authorized July 10, 1918.

Vol. XXX, No. 2 FEBRUARY, 1941

PEPSENCIA with VITAMIN B_1

(thiamine hydrochloride)

PEPSENCIA with VITAMIN B_1 is a new standardized product. Each fluid ounce contains 1000 International units of vitamin B_1.

Put up only in 8 ounce bottles.

Trade Price $12 a dozen.

Literature sent on request

FAIRCHILD BROS. & FOSTER

New York, New York

"I WISH SOMEONE HAD GIVEN ME THIS BOOK WHEN I GRADUATED"

SAY PHARMACISTS ALL OVER THE NATION

This book supplies just the kind of information a pharmacist needs in order to have an intelligent understanding of biological products, their application in therapeutics and merchandising. If you want to boost your biological business get this book today.

BIOLOGICAL PRODUCTS

by Dr. Louis Gershenfeld, P.D., B.Sc. Ph.M.

SOME OF THE SUBJECTS EMBRACED

- Antitoxinum Diphthericum, Serum Sickness
- Antitoxinum Tetanicum
- Antitoxinum Scarlatinae Streptococcicum
- Gangrene and Botulinus
- Serum Antivenenosum, Venins, Venom, etc.
- Antibacterial Serums
- Serum Antimeningococcicum
- Serum Antipneumococcicum
- Antigens
- Vaccinum Typhosum
- Other Bacterial Vaccines, Plague, Cholera, etc.
- Toxinum Diphthericum Detoxicatum, When to Immunize
- Tetanus Toxoid
- Tuberculinum Pristinum
- Bacteriophage and Phagotherapy
- Modified Viruses (Virus Vaccines)
- Vaccinum Rabies
- Yellow Fever
- Poliomyelitis
- Rickettsial Diseases of Man
- Allergens and Sensitization Diseases

$4.00 plus 15c for postage and handling. ORDER NOW.

ROMAINE PIERSON PUBLISHERS, INC.

97 Nassau STREET NEW YORK, N. Y.

Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

A. G. DuMez, Editor, Baltimore, Maryland

Volume XXX *FEBRUARY, 1941* Number 2 Consecutive No. 3

The Toxicity and Trypanocidal Activity of Commercial Neoarsphenamine

*By C. A. Morrell and M. G. Allmark**

The toxicity of neoarsphenamine, as well as of arsphenamine and sulpharsphenamine, offered for sale in Canada is subject to regulations under the Food and Drugs Act of Canada (1). According to the method of assay used in the control of these products in Canada the toxicity is expressed as per cent of the International Standard preparation (2), and in accordance with a recommendation contained in a memorandum from the National Institute for Medical Research (3), batches having a toxicity greater than 120 per cent of the International Standard are not sold in Canada.

Although a test for the therapeutic potency of these drugs is not required under the Canadian regulations, examination of a number of commercial samples by a method recently developed in this laboratory (4) has been carried out. By this test the trypanocidal activity of the samples is also determined as per cent of the International Standard. In the memorandum referred to above (3) it is recommended that the trypanocidal activity of commercial neoarsphenamine should not be less than 80 per cent of the International Standard.

The results of approximately 200 routine assays of neoarsphenamine for toxicity and 76 tests for trypanocidal activity are reported here, and they show, as well as can be done with present methods, the safety and effectiveness of the different preparations offered for sale in Canada.

In addition, a number of observations have been made on the use of dosage-response curves in the assay of arsenicals. Single-dose and multiple-dose methods (4, 5) have been employed, and the regression lines for toxicity and trypanocidal activity have been redetermined and compared with those previously published (2, 4).

A report on the secondary standards used in Canada in routine assays of neoarsphenamine is also included.

MATERIALS AND METHODS

The methods used for the biological tests for toxicity and trypanocidal activity have already been described (2, 4). Both one-dose and multiple-dose methods were employed in this work.

For the one-dose method a single group of rats was injected with a dose of the sample to be tested and another group, of the same sex and similar in respect to age and weight, was injected with a dose of the standard. In all assays 20 to 30 rats were used on each of the two groups. The slope of the dosage-response curve was assumed to be constant and curve numbers (4, 6) were used in calculating the results. For assays carried out up to 1938, curve numbers were taken from a dosage-response curve already published (2), and for those performed since

* The Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.

that time they were computed from a dosage-response curve for sib-mated rats reported in this paper. The difference in the slopes of these curves was so small as to be statistically negligible, however, and curve numbers from either curve would have given satisfactory figures.

For the multiple-dose assays forty to sixty rats were divided into several similar groups. Each group received a different dose; usually two to four doses for each of the standard and sample were used. With this method the slope of the dosage-response curve was determined for every test. Results were computed according to the procedure of Bliss (7, 8).

The rats used were all bred and raised in this laboratory. Their care and the ingredients of the diet were not altered from 1930 to 1939.

RESULTS

The Canadian Standards.—Since 1930 a subsidiary standard for neoarsphenamine has been employed in Canada. The use of a supplementary standard in routine assays conserves the limited supply of the International Standard, and if the relative toxicity and activity of the two standards are known it is a simple matter to express the results in terms of the International Standard. The four successive Canadian Standards employed in the past ten years were batches of commercial material, and were not chosen because they represented the least toxic products on the market, but because they were believed to resemble most nearly the International Standard in their toxicity and physical properties.

The toxicity and trypanocidal value of the Canadian Standards have been determined in relation to the International Standard and the results are shown in Tables I and II.

The toxicities of lots 330, 133 and 336 shown in Table I, approximated 100 per cent, while that of 838 was definitely higher. These figures are weighted mean toxicities obtained from several assays performed on each standard over a period of from two to three years. The limits of error, within which the relationship to the International Standard is known, are given in column 3 of Table I and were computed according to Irwin (9). These limits of error are maximal since, for example, it can be shown to be statistically improbable that Canadian Standard 838 is less than 106 per cent or more than 116 per cent as toxic as the International Standard. The value reported in the table is, of course, the most probable value and is the proper one to use in calculating the toxicity of samples.

The fact that lot 838 is 111 per cent as toxic as the International Standard does not invalidate its usefulness as a comparative standard. A factor of 111 per cent is just as serviceable as one of 100 per cent, except that it necessitates another step in the calculation of results.

In Table II the average trypanocidal activities of only two of the Canadian Standards are shown, since the method used for determining therapeutic potency (4) had not been developed when standards 330 and 133 were employed. The potencies of the standards have been determined by capacity to clear the blood of trypanosomes in a five-hour period and also to cure trypanosomiasis when judged by an observation period of five weeks. The figures given are weighted means and the limits of possible error were calculated as for toxicity (9). It will be noted that the activity of the Canadian Standard determined by negative smears is four or five per cent less than by survivals. This difference was also obtained with most of the commercial samples tested, and could have been due to some characteristic of the International Standard.

Table I.—Toxicity of Canadian Standards for Neoarsphenamine in Terms of the International Standard

Standard Lot No.	Toxicity as Per Cent of International Standard	Maximum Limits of Error from True Value, as Per Cent when $P = 0.95$
330	103	±9
133	99	±8
336	97	±4
838	111	±5

Table II.—Trypanocidal Activity of Canadian Standards for Neoarsphenamine in Terms of the International Standard

Standard	Activity as Per Cent of International Standard		Maximum Limits of Error from True Value, as Per Cent when $P = 0.95$
	Negative Smears[c]	Cures[d]	
336[a]	94		± 6
		99	± 8
Weighted mean	95		± 5
838[b]	78		±12
		82	±10
Weighted mean	80		± 8

[a] Figures obtained by direct comparisons with the International Standard.
[b] Figures obtained by comparison with Canadian Standard 336 and also by comparison with the International Standard.
[c] End point taken as negative blood smears five hours after injection of neoarsphenamine.
[d] End point taken as survival for five weeks after injection of neoarsphenamine.

The limits of error for the determinations of trypanocidal activity are greater than for toxicity because the inherent error of the method is greater, largely due to the more gradual slope of its dosage-response curve (Tables VI and VIII).

The weighted mean of the results in which both smears and survivals are included is also given. For routine assays it does not matter greatly which end point is employed, but it is better, particularly when comparing standards, to use the average from both criteria.

The Toxicity and Trypanocidal Activity of Market Samples.—Table III shows the toxicity of some market samples of neoarsphenamine. Two hundred assays are reported in all, and the products of manufacturers in England, France, the United States, Germany and Canada are included.

The figures given in column 3 of the table are the average toxicities of the different batches of neo-

arsphenamine submitted by the manufacturers for test. The standard deviation represents the variations of the individual determinations from the mean, and includes the errors of the method as well as the variation in the toxicity of different batches. The error of a toxicity assay as measured by its standard error when 40 to 50 rats are used is about ±6 per cent,[1] and is about equal to the variation in the results shown for products of manufacturers 1, 2, 3 6 and 7. It appears, therefore, that the toxicity of batches produced by these companies is quite uni orm. Variability is apparent, however, n the products of manufacturers 4, 5, 8 and 9 since the standard deviation of the individual results exceeds the standard error of the method.

Table III.—Toxicity of Neoarsphenamine Offered for Sale in Canada

Manufacturer	Number of Determinations	Average Toxicity as Per Cent of International Standard	Standard Deviation
1	16	80	7.9
2	34	85	6.9
3	32	85	7.3
4	5	90	13.1
5	49	91	13.0
6	6	100	8.5
7	11	102	5.8
8	20	103	10.3
9	27	115	10.1

In Table IV the average trypanocidal activity of neoarsphenamine from four manufacturers is shown. The limited number of samples assayed in each case makes it impossible to show that the standard deviations are significantly greater than could be anticipated by the error of the method, which is about ±11 per cent[2] when 40 to 50 rats are used, in contrast to the ±6 per cent for toxicity tests. For this reason it cannot be said categorically that there was a variation in the activities of the different batches of any brand.

Table IV.—Trypanocidal Activity of Neoarsphenamine Offered for Sale in Canada

Manufacturer	Number of Determinations	Average Activity as Per Cent of International Standard: Negative Smears[a]	Cures[b]	Standard Deviation
3	10	138		19.6
	7		140	10.3
7	7	96		17.2
	4		100	7.9
9	13	81		8.5
	8		97	11.9
5	15	72		8.9
	12		77	10.4

[a] End point taken as negative blood smears five hours after injection of neoarsphenamine.
[b] End point taken as survival for five weeks after injection of neoarsphenamine.

[1] Computed by equations 39 and 42 (9) for responses between 20 and 80 per cent.
[2] Computed by equations (39) and (42) (9) for responses between 20 and 80 per cent.

In Table V the therapeutic ratio and the therapeutic index for four brands of neoarsphenamine and two of the Canadian Standards are given. The therapeutic ratio is the average activity divided by the average toxicity, when both values are given as per cent of the International Standard. The therapeutic index (or curative ratio) is the ratio of the dose which killed 50 per cent of the rats to the dose curing 50 per cent of those infected with trypanosomes, and is a factor customarily used for assessing the usefulness of drugs. The therapeutic index may vary from colony to colony, depending on the resistance of the rats and trypanosomes to neoarsphenamine, but the therapeutic ratio, which depends only on the relationship of the sample to the International Standard, will be constant despite colony variations in resistance. The therapeutic ratios of the 5 brands of neoarsphenamine were found to vary from 0.76 to 1.64 and these differences emphasize the superiority of some brands of neoarsphenamine when judged by the biological tests used in this laboratory.

Table V.—Ratio of Trypanocidal Activity to Toxicity of Some Brands of Neoarsphenamine Sold in Canada

Manufacturer	Average Toxicity as Per Cent of International Standard	Average Activity[a] as Per Cent of International Standard	Therapeutic Ratio: Average Activity / Average Toxicity	Therapeutic Index
3	85	139	1.64	27
7	102	98	0.96	20
5	91	74	0.81	18
9	115	87	0.76	15
Canadian Standard 336	97	95	0.98	
Canadian Standard 838	111	80	0.72	

[a] The weighted mean of results given in Table IV for both end points.

The Slope of the Dosage-Response Curves and the Design of Tests.—Dosage-response curves for both the toxicity and trypanocidal tests have already been published (2, 4). After 1938 all of the animals used for routine tests were the offspring of brother-sister mating, and it was desirable to determine what effect had been produced by closer inbreeding on the slope of the dosage-response curve. Actually this type of breeding was deliberately adopted in an attempt to steepen the curve, and thus increase the accuracy of tests with a given number of animals (4). Seventy-three assays of the three-dose type were carried out during 1938–1939 and the slope and position of the regression lines computed by the usual methods (7, 8). Since there was no significant difference in their slopes,[3] the lines from individual

[3] Chi-square for b determined from equation (20a) (8).

tests were combined to produce the figures for the composite curves shown in Table VI. This table also compares the slope of the combined lines obtained in 1931–1932 from random-mated rats, with those from sib-mated animals in 1938–1939. The inbreeding has produced a slight increase in slope which, although not significant, does indicate that there is less individual variation in the response to neoarsphenamine.

Table VI.—Slope of the Regression Lines which Show Dosage-Mortality Relationship for the Determination of the Toxicity of Neoarsphenamine Using Albino Rats

Date	Type of Mating	b Slope of Line Weighted Mean	Standard Error	Material
1931–1932	Random	13.7	0.79	Standards and samples
1938–1939	Brother-Sister	15.3	0.93	Standards and samples
1938–1939	Brother-Sister	15.0	1.60	Standards only

Table VII.—Difference in the Slope of the Regression Lines for the Toxicity of Neoarsphenamine Due to the Sex of the Rats

Date	Sex	Type of Mating	b Slope of Line Weighted Mean	Standard Error
1931–1932	Males	Random	13.0	0.95
1931–1932	Females	Random	15.4	1.40
1938–1939	Males	Brother-Sister	13.7	1.68
1938–1939	Females	Brother-Sister	16.0	1.11

[a] Results in each group were obtained from both standards and samples.

Table VIII.—Slope of Regression Lines Showing Dosage-Response Relationship in the Determination of the Trypanocidal Value of Neoarsphenamine Using Albino Rats

Sex of Rats	Type of Response	b Slope of Line Weighted Mean	Standard Error	Type of Breeding
Females	Smears[a]	11.3	1.11	Random
	Survival	6.2	0.60	1935–1936
Males	Smears[a]	7.5	0.48	
	Survival	10.4	0.62	
Females	Smears[a]	8.2	0.79	Brother-Sister
	Survival	7.9	0.98	1938–1939
Males	Smears[a]	8.2	0.61	
	Survival	10.5	0.80	
For brother-sister mating; all tests. Weighted mean		8.7	0.44	

[a] The negative smears end-point.

The figures given in Table VII show the influence of sex on the slope of these curves. Female rats are more uniform in their reaction to neoarsphenamine than males, but the difference is slight.

In Table VIII the regression coefficients for the trypanocidal activity obtained in 1935–1936 are compared with those from the sib-mated rats in 1938–1939. There is a slight but not significant increase for the sib-mated rats in all except the females with negative smears. Since no significant difference[4] can be shown in the four values for b determined in 1938–1939, a weighted mean for all tests has been computed and found to be 8.7 ± 0.44.

DISCUSSION

Assays of Commercial Samples.—The toxicities of the products of nine different manufacturers averaged from 80 to 115 per cent of the International Standard (Table III), and there can be no doubt that some neoarsphenamines are better than others as judged by these tests. There is no real difference in the average toxicities of the brands 1 to 5, but those of 6 to 9 are significantly greater. In fact, the average toxicity of the product of manufacturer 9 approaches the upper limit approved by the National Institute for Medical Research (3).

Another important point in estimating the reliability of the products is the uniformity of different batches. This is indicated by the size of the standard deviations shown in Table III. Here again the products of manufacturers 1, 2 and 3 were among the best. There could have been little real variation in the toxicity of batches submitted by these manufacturers since the error of the method would allow an apparent difference of ± 6 per cent in determination on the same material. On the other hand, manufacturers 4, 5, 8 and 9 were not so successful in producing neoarsphenamine with uniform toxicity. The evidence in Table III indicates that brands 1, 2 and 3 are definitely superior in that their toxicity is low and there is little variation from lot to lot.

Neoarsphenamine having a low toxicity need not necessarily have a low trypanocidal activity. Most of the brands assayed in this laboratory which showed a low toxicity had a high trypanocidal potency, and the most toxic were usually, although not invariably, the least active. This relationship is shown by the figures of Tables III and IV. Manufacturer 3 prepared a product

[4] See footnote No. 3, page 35.

with uniformly low toxicity and it was at the same time the most potent of those examined. In addition, one batch of neoarsphenamine from manufacturer 1 had a therapeutic activity of 120 per cent and a toxicity of 79 per cent of the International Standard. The most toxic product examined was that of manufacturer 9, and its activity was among the lowest. Number 7 stood in an intermediate position both as to toxicity and trypanocidal activity, being practically equivalent to the International Standard in both. The Canadian Standards also showed this relationship; Lot No. 838 was more toxic and less active than Lot No. 336 (Tables I and II).

It should be noted that neoarsphenamine 5 is an exception, and although relatively non-toxic has, on the average, a trypanocidal activity less than 80 per cent of the International Standard, which is lower than that recommended as a minimum (3).

Methods of Assay.—Single-dose and multiple-dose variations of the methods for toxicity and trypanocidal activity have been employed in this work. The advantages and disadvantages of each of these methods of arranging the doses have been discussed elsewhere (4, 5, 10). The theoretical basis for the argument for the use of the one dose method centers largely around whether or not the slope of the dosage response curves remains constant. In routine assays the figures obtained for the slope will differ from test to test, because the individual variation in the whole colony of rats to the treatment cannot be determined exactly from the necessarily small samples of animals used each time. However, it is possible to decide whether or not the differences observed in the values of b computed from the individual assays are due only to this sampling variation (8). The necessary computations for this purpose have been done with the regression lines used in this work and, as no significant differences could be shown among them, they were combined to give composite regression lines with the slopes shown in Tables VI, VII and VIII.

These values for b, acquired from a large number of animals and separate tests, are much closer approximations to the true values for our colony than those obtained with the few rats used in each routine test. The error contributed by uncertainty in the determined value of the slope is consequently reduced by using the composite b (Tables VI, VII and VIII) in calculating the results of assays.

The use of one dose and a composite b is satisfactory in these methods since the slopes cannot be shown to change significantly from time to time. The simplicity of the one-dose technique, particularly in the calculation of results, is a strong recommendation in its favor, especially in routine assays where a large number of tests must be done each week (10). It is not advisable, however, to adopt this method until experience gained with the animals and drugs used has indicated that it is feasible, and it should not be employed when investigating new substances or in research problems.

Steeper dosage-response curves yield more accurate assays. They imply less individual variation in the test animals, and every effort consistent with economy of labor and material, should be made to increase their slopes. Individual variation may be reduced by maintaining a satisfactory and constant environment, and by close inbreeding.

Since the environment appeared to be satisfactory and could be kept constant, an attempt was made to increase b by closer inbreeding of the rats. It was hoped that the results would indicate the influence of the genetic factor in individual variation to a drug. The same colony of rats was employed as in 1931–1932 together with the same diet and care. Matings, however, were all brothers to sisters and the figures for 1938–1939 reported in this paper were obtained with rats which had been inbred for at least six successive generations, and whose origin could be traced to one pair. Tables VI and VII show that there was a slight increase in b which, however, could not be proved to be statistically significant. It was concluded that the accuracy gained did not justify the somewhat greater work necessary for this type of mating.

It is probable, of course, that the rats used

in 1931–1932 had been inbred for some time before they were received in this laboratory in 1928. From then until 1935 they were mated in a random fashion by selecting healthy stock without regard to relationship.

In spite of the failure to significantly increase uniformity in our colony by this means, it has been definitely shown by others that considerable improvement in accuracy results from the use of a carefully bred and tended stock of animals (5, 11).

The slopes of the regression lines for sulpharsphenamine, arsphenamine and mapharsen have been computed for a few assays using male rats in the majority of tests, and are given here as a matter of interest. The average values of b were as follows; for sulpharsphenamine 9 ± 2.1, for arsphenamine 10 ± 1.3 and for mapharsen 9 ± 0.97.

SUMMARY

1. Two hundred tests for the toxicity, and seventy-six tests for the trypanocidal activity of commercial samples of neoarsphenamine are reported. Toxicity and trypanocidal activity are expressed in terms of the International Standard Neoarsphenamine.

2. Differences are shown to exist in the toxicity and activity of the products of different manufacturers.

3. The least toxic neoarsphenamines frequently have the highest trypanocidal activity, as judged by these assays.

4. Some brands are more variable from batch to batch in their toxicity than others.

5. The toxicity of four lots of Canadian Standard Neoarsphenamine, and the trypanocidal activity of two lots are reported.

6. A one-dose method of assay, using curve numbers in calculating results, may be employed with the rats used in this laboratory.

7. The effect of inbreeding on the slope of the dosage-response curve is demonstrated.

REFERENCES

(1) Regulations under the Food and Drugs Act 1934, Department of Pensions and National Health, Ottawa, Canada.

(2) Morrell, C. A., and Chapman, C. W., *J. Pharm Exp. Therap.*, 48 (1933), 391.

(3) League of Nations, Health Organization; Memorandum on the International Standards for Neoarsphenamine and Sulpharsphenamine, M35, Department of Biological Standards, National Institute for Medical Research, London, July 1936.

(4) Morrell, C. A., Chapman, C. W., and Allmark, M. G., *J. Pharm. Exp. Therap.*, 64 (1938), 14.

(5) Gaddum, J. H., Medical Research Council, Special Report Series No. 183, 1933.

(6) Trevan, J. W., *Pharm. J.*, 117 (1926), 439.

(7) Bliss, C. I., *Ann. Appl. Biol.*, 22 (1935), 134.

(8) Bliss, C. I., *Ibid.*, 22 (1935), 307.

(9) Irwin, J. O., *J. Roy. Statistical Soc.*, (Supplement), 4 (1937), 1.

(10) Allmark, M. G., and Morrell, C. A., JOUR. A. PH. A., 30 (1941), 1.

(11) Durham, F. M., Gaddum, J. H., and Marchal, J. E., Medical Research Council, Special Rep. Series No. 128, 1929.

Intravenous Toxicity of Heparin-Sodium Sulfapyridine Combinations and Protective Action of Barbiturates*

By N. A. David, N. M. Phatak, H. Donnell and H. Vehrs†

INTRODUCTION

Murray and Best (1) first demonstrated the usefulness of heparin in the prevention of post-operative thrombosis. Since then studies on the use of heparin in subacute bacterial endocarditis have also been reported (2, 3). In this condition it would seem desirable to give one of the newer chemotherapeutic agents simultaneously with the intravenous administration of heparin in order that the blood stream may be cleared of the invading micro-organisms and preventing at the same time further deposition of thrombotic masses on the heart valves. However, no experimental information has thus far been reported indicating the safety of such a procedure.

EXPERIMENTAL

Prior to the clinical use of heparin and sodium sulfapyridine mixtures intravenously in cases of

* Read before the Scientific Section of the AMERICAN PHARMACEUTICAL ASSOCIATION, Richmond meeting, 1940.

† From the Department of Pharmacology, University of Oregon Medical School, Portland, Oregon.

subacute bacterial endocarditis, we have studied the safety of such a procedure in seven experimental dogs. These animals were given intravenously 5 per cent solution of sodium sulfapyridine in either physiological saline or in molar sodium lactate (Hartmann's solution), and mixed with 2 cc. heparin (2000 units) per 100 cc. of the mixture. Since the usual recommended therapeutic dose of sodium sulfapyridine for man is 60 mg. per Kg. it would seem that 10 times this amount should show definite toxic manifestations in experimental animals; therefore, we injected our dogs with 0.6 Gm. per Kg. of sodium sulfapyridine plus 2 cc. of heparin added per 100 cc. of the mixture. Solutions were warmed to body temperature and injected by gravity at the rate of 2–3 cc. per minute. The results of these experiments showing the levels of the blood sulfapyridine and the plasma-CO_2 values for several hours after injection and the response shown by the animal are summarized for 6 of the 7 dogs in Table I. No blood chemistry studies were made on the first dog treated; observations on this animal are reported in the protocol below.

PROTOCOL

February 8, 1940 Dog No. 1, Spotty Male, 9.1 Kg.

11:40 A.M. Solution made under aseptic conditions as follows:

Heparin Solution (1000 units per cc.)	4.00
Sodium sulfapyridine	10.00
Physiological Saline *q. s. ad*	200.00

8:00 P.M. Solution inspected, appeared clear; warmed to 37 degrees C.

8:15 P.M. Intravenous injection commenced and continued at rate of 2–3 cc. per minute by gravity drip.

8:45 P.M. Salivation and occasional retching noted.

9:00 P.M. Injection of 110.0 cc. of the 5% sodium sulfapyridine-heparin in saline solution completed. Total amount sodium sulfapyridine given, 5.50 Gm. (0.6 Gm. per Kg.)

9:02 P.M. Vomited, defecated and urinated.

9:10 P.M. Sudden onset of severe clonic convulsion with some opisthotonus characterized by extension of limbs with jerking movements and constant snapping of jaws; lasted for 4 minutes until ether applied.

9:14 P.M. Under light ether anesthesia, spasmodic convulsions continue with less severity, jerking movements of limbs at rate of 40 to 51 per minute.

9:18 P.M. Dog completely anesthetized, convulsions cease, ether removed.

9:27 P.M. Second convulsion began suddenly; ether applied at once to point of anesthetization and continued until 9:40 P.M. Convulsion disappeared under anesthesia.

9:40 P.M. Rectal temperature, 39.2 degrees C.

9:45 P.M. Third convulsion began, less intense than previous; ether anesthesia started 1 minute after convulsion began and light anesthesia continued three minutes until convulsion ceased.

10:00 P.M. Fourth convulsion, less intense; light ether anesthesia given for several minutes. Dog relaxed and continued to sleep after anesthesia removed.

11:00 P.M. Dog has continued sleeping, completely relaxed. No further convulsions appeared up to 12:30 P.M. when observations were concluded.

February 9, 1940

8:00 A.M. Dog appears well, drinks warm milk and water. Returned to animal quarters. Survived.

The most constant side effects noticed after the oral or intravenous administration of toxic doses of sodium sulfapyridine are nausea, vomiting, generalized clonic and tonic convulsions, acidosis, drug fever, coma and death. Moderate or even therapeutic amounts cause nausea, salivation, vomiting and often mild jerky muscular tremors. Since we administered the mixtures intravenously, the toxic effects we noted must be attributed to central nervous system stimulation rather than to local gastric irritation or to a reflex therefrom. In all animals either repeated administrations of ether inhalation or a single parenteral injection of 15 to 20 mg. per Kg. of pentobarbital was sufficient to prevent the convulsive seizures and to spare the muscular exhaustion of the animals. However, ether administration alone failed to completely protect the animals. Intraperitoneal injection of the above amounts of sodium pentobarbital given 20 to 30 minutes previous to or soon after completion of the injection of sodium sulfapyridine not only minimized the toxicity of the drug but completely protected the animals from convulsive seizures and certain death. When sodium pentobarbital was given in conjunction with intravenous administration of sodium sulfapyridine-heparin mixtures, the dogs invariably showed a complete recovery to normal in less than 24 hours. This use of the barbiturate (a "short-acting" one) is not only spectacular but a definite life saving measure and should be of therapeutic value in counteracting the toxic effects of sulfanilamide derivatives when given to man.[1]

[1] Adriani (4) has recently reported that the use of sub-anesthetic doses of several barbiturates in rats receiving sulfanilamide serves to increase the toxicity of the latter drug. It is to be noted that we employed only hypnotic doses of the barbiturate and used sulfapyridine rather than sulfanilamide.

Table I.—Response of Dogs and Changes in the Blood Sulfapyridine and Plasma CO_2 Levels Following Intravenous Injections of Sodium Sulfapyridine-Heparin Mixtures

Dog No.		Fasting	Time: ½ hr.	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	8 hrs.	Drugs Given and Response of Dogs
2	Sulfapyrid.		36.0	42.8	48.1	43.5	43.5		38.8		0.6 Gm./Kg. 5% Sod. Sulfapyr. in saline. No Heparin. 25 mg./Kg. Pentobarbital given I.P. 1½ hrs. previously. No reaction. Died 48 hrs. later of pneumonia.
	Plasma CO_2	33.6	27.9	38.0	50.4	53.7	57.3		51.5		
3	Sulfapyrid.		53.0	50.0	49.2						0.6 Gm./Kg. 5% Sod. Sulfapyr. in saline with Heparin. Ether only to prevent convulsions. Died 2 hrs. after start of injection; excessive rise of temperature.
	Plasma CO_2	60.5	58.5	68.3	66.2						
4	Sulfapyrid.		46.3	47.7	46.3	47.0	44.9				0.6 Gm./Kg. 5% Sod. Sulfapyr. in glucose-saline with Heparin. Mild convulsions controlled by ether but necessary to give 20 mg./Kg. Pentobarbital after 2 hrs. O.K. Survived.
	Plasma CO_2	60.5	48.2	45.0	68.3	54.4	55.3				
5	Sulfapyrid.		40.0	41.4	40.7	37.0	33.9		28.6		0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin followed in ½ hr. by 14 mg./Kg. Pentobarbital I.P. O.K. Survived.
	Plasma CO_2	50.2	68.8	81.1	70.8	64.8	65.9		58.5		
6	Sulfapyrid.		39.6	33.9	33.1	28.3	27.2	22.4			0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin followed in ½ hr. by 14 mg./Kg. Pentobarbital I.P. O.K. Survived.
	Plasma CO_2	60.0	70.8	62.7	57.6	69.9	60.0	56.4			
7	Sulfapyrid.		38.5	40.9	32.5	31.8	32.5	24.9	23.8	21.4	0.6 Gm./Kg. 5% Sod. Sulfapyr. in molar sod. lactate with Heparin. 15 mg./Kg. Pentobarbital I.P. ½ hr. before injection started. O.K. Survived.
	Plasma CO_2	58.5	75.9	60.5	55.6	59.6	65.9	67.9	62.7		

Use of molar sodium lactate with heparin-sodium sulfapyridine mixtures materially alleviates acidosis and serves as an efficient buffer as evidenced by plasma CO_2-capacity determinations. In dogs the use of molar sodium lactate maintains a plasma CO_2 level between 60 to 80 volumes per cent, which, in its absence, remains at 40 to 50 volumes per cent. It alone, however, does not prevent the appearance of toxic effects of sodium sulfapyridine but lessens the severity and mitigates the after effects.

A mixture of sodium sulfapyridine and heparin remains stable in solution for at least 6 to 10 hours and often up to as long as a week depending on the condition of storage. Heparin and sodium sulfapyridine mixtures are not stable in either glucose or glucose-saline vehicle.

CONCLUSIONS

From our studies we can say that heparin-sodium sulfapyridine mixtures may be safely administered intravenously as a continuous drip.

Use of sodium pentobarbital, orally or parenterally in hypnotic doses, either previous to treatment or simultaneously with it, is not only beneficial but advisable as an adjunct to reduce the untoward manifestations of sodium sulfapyridine therapy.

Use of molar sodium lactate is recommended in preference to either physiological saline or glucose as a vehicle for the parenteral administration of heparin-sodium sulfapyridine to counteract the acidosis.

We wish to thank Dr. K. K. Chen, Lilly Research Laboratories, Indianapolis, Indiana, for sending us generous supplies of sodium sulfapyridine and sodium pentobarbital. The heparin we used was obtained in 10 cc. vials (1000 units per cc.) from the Connaught Laboratories, Toronto, Canada.

REFERENCES

(1) Murray and Best, *Jour. A. M. A.*, 110 (1939), 118.

(2) Kelson and White, *Ibid.*, 113 (1939), 1700.

(3) Friedman, Hamburger and Katz, *Ibid.*, 113 (1939), 1702.

(4) Adriani, *Jour. Lab. and Clin. Med.*, 24 (1939), 1066.

Fritz Pregl (1869–1930) was awarded the Nobel Prize for Chemistry in 1923 for his work on the micro-analysis of organic substances, just ten years after he explained the fundamentals of his methods at a meeting of naturalists and physicians in Vienna.

Bacteriostatic Properties of Phenylacetic Acid

Effect of Ortho-Substituents on*

By C. F. Feasley and B. H. Gwynn, with Ed. F. Degering and P. A. Tetrault

The use of acids as antiseptics is a natural consequence of the fact that the growth of bacteria *in vitro* is self-limited and that organic acids, produced from the medium in which bacteria are growing are one of the limiting factors. This was indicated by Taylor (28) as early as 1917.

Mineral acids have bacteriostatic action, which is inversely proportional in general to the p_H of the solution, but organic acids exhibit a high degree of specificity. There are some rather definite correlations between structure, chemical and physical properties, and bacteriostatic and bactericidal activity. These have been considered in some detail in previous papers, but are briefly summarized in the following paragraph.

Bacteriostatic and bactericidal activity is found to parallel rather closely with (1) an increase in the hydrogen ion concentration of the medium (5, 12, 14, 31), (2) an increase in the oil-water distribution coefficient (3, 4, 10, 15), (3) an increase in adsorption on activated charcoal (3, 10, 15) and (4) a lengthening of the carbon chain (3, 6, 10, 15, 20).

The effect of unsaturation has not been clearly established. In some few cases, particularly with the phenols, unsaturation definitely enhances the bacteriostatic properties (16). With acids this effect is not so apparent. In this series unsaturation may even show slight inhibition on the effectiveness of the bacteriostatic agent. This may be attributed in part to the susceptibility of bacteriostatic and bactericidal reagents to unstable organic compounds (8).

Closely allied to this is the relation between chemical stability and bacteriostatic activity. This has been investigated in the case of maleic and fumaric acids, and the stabler isomer (fumaric acid) has been found to be a more effective bacteriostatic agent (8).

The effect of varying a substituent in an aromatic nucleus, as well as varying the substituent in any given position on the nucleus (4, 19) has been studied but not generally enough to permit of many definite conclusions. In general, however, *p*-substituted compounds have been found to be more effective against bacteria than have the corresponding *o*-substituted isomers.

In this research a study has been made of certain *o*-substituted phenylacetic acids, and the bacteriostatic properties of these compounds compared with those of the corresponding members of the *p*-series (4). A few other acids have been included in the group in an effort to obtain further information about the effect of unsaturation, position isomerism, and *cis-trans* isomerism on bacteriostatic activity.

EXPERIMENTAL

A number of *o*-substituted phenylacetic acids have been prepared by variations in methods available in the literature. *o*-Nitrophenylacetic acid was prepared by a modified Reissert method (22, 24) in which ethyl oxalate was condensed with *o*-nitrotoluene in the presence of sodium ethoxide to give the sodium salt of *o*-nitrophenylpyroracemic acid. This product was oxidized with hydrogen peroxide, and the reaction mixture acidified to give *o*-nitrophenylacetic acid.

o-Aminophenylacetic acid was obtained by the reduction of *o*-nitrophenylacetic acid with hydrogen, in the presence of colloidal platinum and ethanol, in an Adams reductor (1). The general procedure follows the method described by Neber (23).

o-Hydroxyphenylacetic acid was prepared from the methyl ether of salicylaldehyde (17). This ether was converted to the bisulfite addition compound, and then treated with a saturated aqueous solution of potassium cyanide. The resulting cyanohydrin was extracted with ether, the extracts dried, decolorized with Norite and the ether recovered.

---

* An abstract of portions of theses submitted to the Faculty of Purdue University by B. H. Gwynn (1939) and C. F. Feasley (1940) in partial fulfilment of the requirements for the degree of Master of Science.

This project was sponsored by the Mallinckrodt Chemical Works, St. Louis, Mo.

This is the eighth of a series of papers by Ed. F. Degering and collaborators on the effect of structure and the p_H of the media on the bacteriostatic and bactericidal properties of alkanoic acids and commercial antiseptics. The others appeared in: *Ind. Eng. Chem.*, 30 (1938), 646, JOUR. A. PH. A., 27 (1938), 865, *Ind. Eng. Chem.*, 31 (1939), 742, JOUR. A. PH. A., 28 (1939), 514, *Ind. Eng Chem.*, 32 (1940), 996, *J. Chem. Educ.*, in publication, *Proceedings Ind. Academy of Science*, 49 (1940), 42.

The colorless cyanohydrin residue was refluxed for 2 hours with 20 ml. of 57% hydrogen iodide in the presence of red phosphorus, and the mixture poured into a solution of sodium bisulfite. The excess phosphorus was removed by filtration, washed with ether, and the filtrate extracted with ether to obtain the crude acid. This was purified by extraction with a 10/1 chloroform-ether mixture.

The *o*-bromophenylacetic, the *o*-chlorophenylacetic and the *o*-methylphenylacetic acids were all prepared by treating the appropriate benzyl halides with sodium cyanide to give the corresponding substituted benzyl cyanides. The cyanides were then converted to the corresponding acids. It was found that acid hydrolysis gave better yields of the *o*-bromophenylacetic and *o*-chlorophenylacetic acids, whereas alkaline hydrolysis gave the best yields of the *o*-methylphenylacetic acid.

Cinnamic acid was prepared according to Knoevenagel (18).

Phenylpropiolic acid was obtained by converting cinnamic acid to the dibromide (9) and subsequent removal of two equivalents of hydrogen bromide (21).

Hydrocinnamic acid was prepared by the reduction of sodium cinnamate with sodium amalgam, acidification, isolation and crystallization from petroleum ether (9).

The *cis-o*-methoxycinnamic and the *trans-o*-methoxycinnamic acids were obtained by the method of Reychler (25).

The *o*- and *p*-nitrocinnamic acids were prepared by the method of Tiemann and Opperman (30), except that mixed acid was used in the place of fuming nitric acid.

m-Nitrocinnamic acid was synthesized by the method of Thayer (29).

Furylacrylic acid was prepared similarly (29) but the mixture was heated at 165–170° for seven hours.

Furylpropanoic acid was obtained by the reduction of furylacrylic acid with sodium amalgam in water (9).

2-Iodo-4-nitrophenylacetic acid, a new compound, was prepared by dissolving 6 g. (0.033 mols) of *p*-nitrophenylacetic acid in 50 ml. of glacial acetic acid in a 250-ml. three-necked round bottom flask equipped with a dropping funnel, a reflux condenser and a mechanical stirrer (11, 26). To this was added 5 g. of iodine and then dropwise, during the period of refluxing, 12.5 ml. of nitric acid (sp. gr. 1.41). Refluxing was continued until there was no evolution of the oxides of nitrogen or evidence of iodine vapors.

The reaction mixture was poured into ice water to precipitate a yellow solid, which was filtered off. This product was dissolved in 95% alcohol, decolorized with Norite, and recrystallized three times to give 3 Gm. of a light yellow crystalline powder, which melted at 236°.

The determination of a neutral equivalent on this acid clearly indicated that it contained only one iodine atom per molecular weight. Since both of the initial groups in the ring were directing to the 2-position, it is assumed that the product obtained was 2-iodo-4-nitrophenylacetic acid.

2-Iodo-4-aminophenylacetic acid, a new compound, was obtained by dissolving 3 g. of 2-iodo-4-nitrophenylacetic acid in 150 ml. of 95% ethyl alcohol in an Adams reductor, and then reducing with hydrogen in the presence of colloidal platinum. After reduction, the catalyst was filtered off, the solution decolorized by treatment with Norite and the solvent removed by vacuum distillation at room temperature. The residue was recrystallized twice from hot 95% ethyl alcohol to give a fine, crystalline solid with a m. p. of 184°.

The Development of a New Test Technique.—Because of the limited solubility of these acids in pure distilled water, no satisfactory bacteriostatic tests could be obtained. A need immediately arose for a new non-toxic medium in which the acids were sufficiently soluble.

The medium that filled the needs more closely than others tried was a dilute solution of 1,4-dioxan. One part of 1,4-dioxan in 10 parts of water prevents growth, but 1 part of 1,4-dioxan to 15 parts of water allows growth of both *Staphylococcus aureus* and *Escherichia coli*.

It has been used with success on various acids that were not sufficiently soluble in pure distilled water to give tests. The bacteriostatic tests of a few of these acids are given in Table II along with the bacteriostatic tests of certain acids in both distilled water and in the 1,4-dioxan medium.

Preparation of Solutions for the Bacteriostatic Tests.—Since previous works in this laboratory have shown that the hydrogen-ion concentration is an important factor in the bacteriostatic property displayed by any solution, it was necessary to make the solutions up to a definite p_H.

In making up the solutions, the proper amount of the acid was transferred to a 25-ml. volumetric flask where it was dissolved in 15 ml. of a 1,4-dioxan-water mixture. The solutions were then adjusted to a p_H of 5 by the addition of dilute hydrochloric acid or dilute sodium hydroxide. A Universal potentiometer assembly designed by Goodhue (13) and modified by Swank (27) was used in adjusting the solutions.

Accurately prepared buffer solutions were used in checking the glass electrode both before and after each period of use. The quinhydrone glass electrode and the calomel cell were rinsed well with distilled water and dried before insertion into a new acid solution.

After adjusting the solutions, they were returned to the volumetric flask, the electrodes washed well with water adjusted to a p_H of 5, the washings being added to the solution and the flask diluted to the 25-ml. mark with distilled water adjusted to the desired p_H of 5. The p_H of this solution was rechecked, a drop or two of acid or base being added

as needed and the solution returned to the flask and stoppered.

Interpretations of the Bacteriostatic Tests.—The results of the bacteriostatic tests for the ortho-substituted phenylacetic acids and a few related compounds are given in Table I.

None of the simple ortho-substituted phenylacetic acids were more effective than the parent compound, phenylacetic acid. Four related compounds were, however, more effective than phenylacetic acid. These acids were diphenylacetic acid, *N*-methyl-*p*-amino-phenylacetic acid, 2-iodo-4-nitrophenylacetic acid and 2-iodo-4-aminophenylacetic acid.

The members of the *ortho*-series tested in this study fall in the decreasing order of hydroxy, chloro, bromo, methyl and nitro. In the *para*-series the order is bromo, iodo, chloro, ethyl, nitro and hydroxy (4). Oxindole is much more specific against *Escherichia coli* than against *Staphylococcus aureus*. In the former case it has an activity a little less than that of the *o*-hydroxy-isomer, while in the latter case it was the least active of the compounds tested in this series.

It was found that replacement of an α-hydrogen by a hydroxyl group decreases bacteriostatic properties. Benzilic acid is less effective than diphenyl-

Table I.—Bacteriostatic Tests in 1,4-Dioxan Solution

Name of Compound	pH ±0.1	Highest Killing Dilution[b] *Staph. aureus* Weight	*Staph. aureus* Normality	*Es. coli* Weight	*Es. coli* Normality
2-Iodo-4-nitrophenylacetic acid[c]	5	1/2658	0.00123	1/4430	0.000737
2-Iodo-4-aminophenylacetic acid[c]	5	1/738	0.00490	1/920	0.00393
Phenylacetic acid	5	1/1200	0.00612	1/1200	0.00612
o-Hydroxyphenylacetic acid[d]	5	1/669	0.00984	1/892	0.00737
o-Chlorophenylacetic acid	5	1/396	0.01472	1/584	0.009875
o-Bromophenylacetic acid	5	1/313	0.01495	1/468	0.00994
o-Methylphenylacetic acid	5	1/448	0.01485	1/540	0.01188
o-Nitrophenylacetic acid	5	1/376	0.01467	1/470	0.0172
Oxindole	5	1/381	0.0196	1/888	0.00844
N-benzoyl, *o*-aminophenylacetic acid[c]	5	a		a	
Benzilic acid	5	1/448	0.009825	1/1082	0.00402
Diphenylacetic acid	5	1/1377	0.003422	1/1836	0.002565
Furylacrylic acid	5	1/315	0.02281	1/630	0.0115
Furylpropionic acid	5	1/543	0.01315	1/1086	0.00656
p-Methylphenylacetic acid[e]	5	1/540	0.01232	1/540	0.01232
N-methyl-*o*-aminophenylacetic acid[e]	5	1/1230	0.00490	1/2065	0.00294

[a] The lowest possible dilution did not kill the bacteria.
[b] A control of the 1,4-dioxan solution gave growth in every dilution comparable to test solutions.
[c] These compounds were prepared in part by Carl Gochenour.
[d] This compound was prepared with Julian Dorskey.
[e] These compounds were prepared by D. Herman.

Table II.—Bacteriostatic Tests

Name of Compound	pH ±0.1	Highest Dilution That Inhibits *Staph. aureus* in 10% EtOH[b]	*Staph. aureus* in 1,4-Dioxan[c]	*Es. coli* in 10% EtOH[b]	*Es. coli* in 1,4-Dioxan[c]
cis-o-Methoxycinnamic acid	5	1/522	1/625	1/522	1/625
trans-o-Methoxycinnamic acid	5	a	1/3000	a	1/4000
trans-Cinnamic acid	5	a	1/1000	a	1/1250
Phenylpropiolic acid	5	1/286	1/308	1/286	1/385
Hydrocinnamic acid	5	1/376	1/830	1/576	1/830
o-Nitrocinnamic acid	5	a	a	a	a
m-Nitrocinnamic acid	5	a	a	a	a
p-Nitrocinnamic acid	5	a	a	a	a
p-Nitrocinnamic acid, ethyl ester	5	a	a	a	a

[a] The lowest possible dilution did not kill.
[b] A control of 10% ethanol gave growth in every dilution comparable to test solutions.
[c] A control of 1,4-dioxan gave growth in every dilution comparable to test solutions.

Table III.—Bacteriostatic Tests[b]

Name of Compound	pH ±0.1	Highest Dilution That Inhibits *Staph. aureus* in H_2O	*Staph. aureus* in 1,4-dioxan[c]	*Es. coli* in H_2O	*Es. coli* in 1,4-dioxan[c]
Phenylacetic acid	5	1/1100	1/1200	1/1500	1/1200
p-Chlorophenylacetic acid	5	1/3000	1/3000	1/1100	1/1000
p-Bromophenylacetic acid	5	1/4000	1/3000	a	1/800
p-Iodophenylacetic acid	5	1/4000	1/648	a	1/810
p-Hydroxyphenylacetic acid	5	a	1/650	a	1/640
p-Ethoxyphenylacetic acid	5	1/1000	1/460	a	1/664
N-Acetyl-*p*-aminophenylacetic acid	5	a	1/500	a	1/625

[a] The lowest dilution gave positive growth because of the limited solubility in water medium.
[b] These compounds were prepared by W. A. Bittenbender (4).
[c] A control of the 1,4-dioxan solution gave growth in every dilution comparable to test solutions.

acetic acid. Either free alcoholic or free phenolic hydroxyl groups have been found by other workers (*23a*, *26a*) to decrease the bactericidal activity. The replacement of an α-hydrogen by the benzene nucleus was found to increase the effectiveness of the bacteriostat. This is evidenced by the activity of phenylacetic and diphenylacetic acid.

The substitution of an iodine atom in the 2-position of *p*-nitrophenylacetic acid greatly enhanced the bacteriostatic properties against both organisms. The iodine-containing compound is more effective against *Escherichia coli* whereas *p*-nitrophenylacetic acid, which was tested by Bittenbender (4), is more effective against *Staphylococcus aureus*. Both 2-iodo-4-aminophenylacetic acid and *p*-aminophenylacetic acid (4) were more effective against *Escherichia coli*.

In all cases except the hydroxy derivatives of phenylacetic acid, the *para*-isomer is superior to the corresponding *ortho*-isomer. The *p*-halogenphenylacetic acids are more effective against *Staphylococcus aureus* than they are against *Escherichia coli*. The reverse is true in the two *o*-halogenphenylacetic acids studied in this series.

Table III shows the results of several compounds prepared by Bittenbender (4) that have been retested in 1,4-dioxan solution. Some of these acids because of their limited solubility in water medium gave positive growth in the lowest possible dilution against both bacteria while other acids were effective only against one organism. A few acids are also included in Tables II and III to compare the bacteriostatic properties in the two media. The test organisms used were *Escherichia coli* and *Staphylococcus aureus*.

In most cases studied the bacteriostats were found to be less effective (disregarding solubility) in the 1,4-dioxan medium than in pure water. This is in harmony with the results of many other workers who have found bacteriostats and bactericides less effective in the presence of organic matter. A brief literature survey on this and closely related effects has been made by one of the writers (*12a*).

Unsaturation is one of the structural changes that has been considered in this report. The saturated isomer is more efficient in the furylacrylic and furylpropionic acid pair. Phenylpropiolic is much less effective than either *trans*-cinnamic acid or hydrocinnamic acid. This, likewise, furnishes evidence that the unsaturated compound is less effective than the saturated compound. Cooper and associates (8) have found that unsaturated acids such as acrylic, crotonic, maleic and fumaric are very susceptible to organic matter. This susceptibility to organic matter may account in part for the lower activity of unsaturated compounds. The position of the double bond is bound to be of importance in any case because of its chance for resonance with the carboxyl group, the benzene nucleus, or both which should change somewhat the properties of the acid being studied.

The phenyl group was found to be more effective than the furyl group in comparing *trans*-cinnamic acid with furylacrylic acid and also hydrocinnamic acid with furylpropionic acid.

SUMMARY

1. Seven *ortho*-substituted derivatives of phenylacetic acid, and 17 closely related compounds were prepared and their bacteriostatic properties tested against *Staphylococcus aureus* and *Escherichia coli*.

2. A solution of 1,4-dioxan was first used as a test medium for antiseptics or bacteriostats. Bacteriostatic tests were completed for seven previously prepared acids that had failed to give tests in other media in which they were less soluble. Oxindole was much more effective against *Escherichia coli* than against *Staphylococcus aureus* while with other compounds the difference was less pronounced.

3. None of the simple *ortho*-substituted phenylacetic acids were as effective as the parent compound, phenylacetic acid. They fell in the following decreasing order: hydroxy, chloro, bromo, methyl and nitro.

4. Phenylpropiolic acid was less effective than either *trans*-cinnamic acid or hydrocinnamic acid. Likewise, furylacrylic acid was less effective than furylpropionic acid.

5. The replacement of an α-hydrogen of diphenylacetic acid by a hydroxyl group was found to decrease the bacteriostatic properties. A phenyl group was shown to be superior to a furyl group and far superior to a hydrogen atom in the α-position.

6. The substitution of an iodine atom in the 2-position of *p*-nitrophenylacetic acid greatly increased its effectiveness.

7. In all cases except the hydroxy derivatives of phenylacetic acid that have been studied the *para*-isomer is superior to the corresponding *ortho*-isomer.

BIBLIOGRAPHY

(1) Adams, Roger and Voorhees, V., *Organic Synthesis*, 8 (1928), 10–16.

(2) Adams, Voorhees and Shriner, *Ibid.*, 8 (1928), 92–99.

(3) Baldinger, L. H., and Nieuwland, J. A., JOUR. A. PH. A., 22 (1933), 711.

(4) Bittenbender, W. A., and Degering, Ed. F., *Ibid.*, 28 (1939), 514–519.

(5) Bittenbender, W. A., Degering, Ed. F., and Tetrault, P. A., *Ind. Eng. Chem.*, 31 (1939), 742.

(6) Calloway, N. O., Gilman, H., and Workman, C. H., *Proc. Iowa Acad. Sci.*, 40 (1933), 81.

(7) Cheeseworth, E. A., and Cooper, H. G., *J. Phys. Chem.*, 33 (1929), 720.

(8) Cooper, E. W., Woodhouse, D. L., and Forstner, G. L., *J. Soc. Chem. Ind.*, 44 (1925), 134–138T.

(9) Cumming, Hopper and Wheeler, "Systematic Organic Chemistry," D. Van Nostrand and Company (1937).

(10) Daniels, T. C., and Lyons, R. E., *J. Phys. Chem.*, 35 (1931), 2049–2060.

(11) Datta, R. L., and Chatterjee, N. R., *J. Am. Chem. Soc.*, 41 (1919), 292–295.

(12) Degering, Ed. F., Tetrault, P. A., Bittenbender, W. A., Feasley, C. F., and Gwynn, B. H., "A Further Study of the Effect of pH on the Bactericidal Properties of Some Commercial Antiseptics," *Ind. Eng. Chem.*, 32 (1940), 996.

(12*a*) Feasley, C. F., "The Effect of Various Factors Other Than Structure upon Bactericidal Action," Term Report in Chemical Engineering 127, Purdue University, March 1940.

(13) Goodhue, L. D., *Iowa State College J. Sci.*, 10 (1935), 7.

(14) Goshorn, R. H., Degering, Ed. F., and Tetrault, P. A., *Ind. Eng. Chem.*, 30 (1938), 646.

(15) Goshorn, R. H., and Degering, Ed. F., JOUR. A. PH. A., 27 (1938), 865–870.

(16) Hurd, C. O., and Parrish, G., *J. Am. Chem. Soc.*, 57 (1935), 1731–1734.

(17) Katschalowsky, A., and Kostanecki, St. V., *Ber.*, 37 (1904), 2347.

(18) Knoevenagel, E., *Ber.*, 31 (1898), 2604.

(19) Labes, R., and Jansen, E., *Arch. expt'l. Path. Pharmakol.*, 158 (1930), 1; *Chem. Abstr.*, 25 (1931), 3899.

(20) Laws, P., *J. Physiol.*, 17 (1894–1895), 360.

(21) Lieberman, C., and Sachse, H., *Ber.*, 24 (1891), 4113.

(22) Mayer and Balle, *Ann.*, 403 (1914), 188–189.

(23) Neber, P. W., *Ber.*, 55B (1922), 826–848.

(23*a*) Overbaugh, S. C., and Sandin, R. B., *J. Am. Chem. Soc.*, 57 (1921), 587–594.

(24) Reissert, Arnold, *Ber.*, 30 (1897), 1036–1043.

(25) Reychler, A., *Bull. Soc. Chim. de France*, (4) 3, 552; *Chem. Zentr.* (1908), 1, 2097.

(26) Robertson, G. R., *Organic Synthesis*, 2 (1922), 59–60; 3 (1922), 11.

(26*a*) Sabalitschka, T. H., *Arch. Phar.*, 269 (1931), 228–246.

(27) Swank, H. W., "A Spectroscopic Study of Colorimetric Methods of Determining Iron," Ph.D. Thesis, Purdue University (1937).

(28) Taylor, K., *Lancet*, 1 (1917), 294–297.

(29) Thayer, F. K., *Organic Synthesis*, 5 (1925), 83–84.

(30) Tieman, F., and Opperman, J., *Ber.*, 13 (1883), 2056–2073.

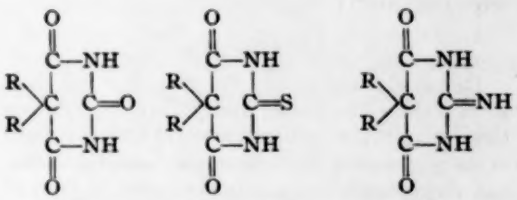
(31) Waterman and Kuiper, *Rec. trav. Chim.*, 43 (1924), 323–325.

The Synthesis of Several Dialkylmalonylguanidines with a Preliminary Note on Their Pharmacology*

By Orville H. Miller† and Louis Fischer‡

In searching for new therapeutically valuable hypnotics of the barbiturate series, much attention has been devoted to replacing the two hydrogen atoms in the 5,5 position of barbituric acid with a wide variety of alkyl, aryl and other radicals. Recently a series of dialkyl thiobarbiturates (2 thio-5,5 dialkylbarbituric acids) have likewise been prepared. Of these several were found to be hypnotics and better adapted to certain uses than the barbiturates.

A few members of a third series in which the urea oxygen is replaced by an imino group (2 imino-5,5 dialkylbarbituric acids) have also been prepared, chiefly for the purpose of converting to the corresponding barbiturate. The chemical relationship of the three series may be best expressed structurally:



Barbiturate | Thiobarbiturate | Iminobarbiturate (Substituted Malonyl Guanidine)

Aside from an early experiment by Fischer and von Mering (1) there has been little interest indicated in the pharmacology of this iminobarbiturate series. Apparently basing their results on a single administration, Fischer and von Mering found dipropylmalonylguanidine to be devoid of any

* Presented to the Scientific Section of the A. PH. A., Richmond meeting, 1940.

† Submitted in partial fulfilment of Master of Science in Pharmacy.

‡ Assistant Professor of Pharmaceutical Chemistry, University of Washington, Seattle, Washington. Suggestion of problem and supervision of preliminary pharmacology by Dr. J. M. Dille, Associate Professor of Pharmacology, University of Washington.

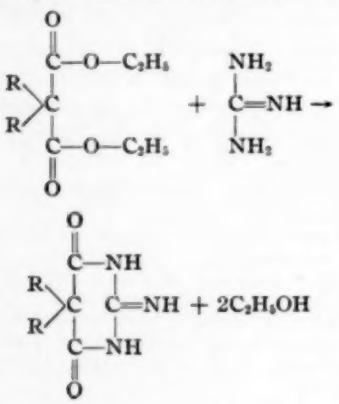
hypnotic or other pronounced pharmacological activity.

Since the soporific action of the thiobarbiturates indicates that the oxygen of the urea group is not an indispensable factor in the barbiturate type of hypnotic, the authors have prepared a series of dialkyl substituted malonylguanidines, namely, diethylmalonylguanidine, ethylisopropylmalonylguanidine, ethyl - *n* - butylmalonylguanidine, ethylisoamylmalonylguanidine and ethylphenylmalonylguanidine and made a preliminary pharmacological study to determine whether or not these derivatives possess any hypnotic properties. Of these diethylmalonylguanidine has been previously prepared by several investigators (2, 3, 4, 5, 6, 7, 8, 9). In addition, monoethylmalonylguanidine (3); dimethylmalonylguanidine (6); dipropylmalonylguanidine (2, 6, 7, 8); ethylpropylmalonylguanidine (5); benzylmalonylguanidine (10); allylmalonylguanidine, diallylmalonylguanidine and allylbenzylmalonylguanidine (11); monomethylmalonylguanidine (isosuccinylguanidine) (12) and unsubstituted malonylguanidine (13, 14) were previously reported.

EXPERIMENTAL

Method of Preparation.—The procedure was similar to that used by Fischer and Dilthey (2). Fifteen Gm. of guanidine hydrochloride (12 Gm. was used in the preparation of ethylisoamylmalonylguanidine and ethylphenylmalonylguanidine) and 25 Gm. of the proper disubstituted malonic ester were heated in a pressure flask, at 80–90° C., in the presence of sodium alcoholate (9 Gm. sodium plus 150 cc. absolute alcohol), for a period of sixty hours. The mixture was dissolved in water, then neutralized with acetic acid, the precipitated dialkylmalonylguanidine filtered off and washed free of chlorides.

Essentially the condensation proceeds as follows:



The compounds were purified by dissolving in warm dilute nitric acid and reprecipitated by adding dilute ammonia until faintly alkaline, avoiding an excess. The precipitate was washed first with water to remove the ammonium nitrate, then with alcohol to remove traces of barbiturates and finally dried at 105° C. On precipitation no crystalline structure was discernible but on cooling a hot alcoholic or aqueous solution, long, slender, colorless, friable needles were obtained.

The purity and percentage yields of each derivative are presented in table form.

Table I.—Yields and Nitrogen Determinations

Substituted Malonylguanidine	Yield Per Cent	Per Cent of Nitrogen by Micro Dumas Method Calculated	Found
Diethyl	60	22.95	23.14
Ethylisopropyl	49	21.31	21.48
Ethyl-*n*-butyl	69	19.90	19.70
Ethylisoamyl	58	18.66	18.58
Ethylphenyl	64	18.18	18.01

General Properties.—The compounds were white in color, non-crystalline in structure and did not melt on heating to 300° C. They were soluble in dilute mineral acids, alkalis, alkali carbonates, very slightly soluble in hot water and hot alcohol and practically insoluble in acetone, chloroform, ether and benzene.

On attempting to prepare hydrochlorides and nitrates according to the method of Fischer and Dilthey (2) the corresponding barbiturates were obtained instead. The conversion to barbiturates took place much more readily in hot hydrochloric acid than with hot dilute nitric acid. The reaction consists of the hydrolytic replacement of the imido group by oxygen, with the formation of ammonia.

Preliminary Pharmacology.—The compounds were prepared for injection by dissolving in a slight excess of alkali, neutralizing until the first appearance of a precipitate and filtering. Intravenous injection into rabbits indicated that ethylisopropylmalonylguanidine, ethyl-*n*-butylmalonylguanidine and ethylisoamylmalonylguanidine produced death apparently by respiratory depression in relatively small doses. Diethylmalonylguanidine and ethylphenylmalonylguanidine were found to be less toxic. While the compounds depress the respiration in doses approaching the lethal dose, there is little evidence of general depression or abolition of reflexes. The evidence obtained in this study is insufficient to form any definite conclusions as to the hypnotic properties of the dialkylmalonylguanidines. Further investigation, using other animals, is now in progress.

SUMMARY

Five dialkylmalonylguanidines, diethylmalonylguanidine, ethylisopropylmalonylguanidine, ethyl - *n* - butylmalonylguanidine, ethylisoamylmalonylguanidine and ethylphenylmalonylguanidine, were pre-

pared by the condensation of the proper dialkylmalonic ester and guanidine hydrochloride, in the presence of sodium alcoholate.

In a preliminary investigation of their pharmacological properties, the compounds were found to produce death by respiratory failure. Sufficient evidence has not yet been obtained to definitely classify the dialkylmalonylguanidines as hypnotics.

BIBLIOGRAPHY

(1) Fischer, E., and von Mering, J., *Die Therapie der Gegenwart*, 44 (1903), 99.

(2) Fischer, E., and Dilthey, A., *Liebigs Ann. Chem.*, 335 (1904), 334–368.

(3) Schering, E., Ger. pat. 174,940, Nov. 15, 1904.

(4) Merck, E., Ger. pat. 235,802, Dec. 1, 1904, and 231,887, Feb. 17, 1906.

(5) Conrad, M., *Liebigs Ann. Chem.*, 340 (1905), 310–325.

(6) Merck, E., Ger. pat. 158,890, Oct. 31, 1903.

(7) Basle Chemical Works, Brit. pat. 5316, March 9, 1908.

(8) Basle Chemical Works, Ger. pat. 204,795, July 30, 1907.

(9) Dox, A. W., *J. Am. Chem. Soc.*, 53 (1931), 1559–1566.

(10) Kast, H., *Ber.*, 45 (1912), 3124–3135.

(11) Johnson, T. B., and Hill, A. J., *Am. Chem. J.*, 46 (1911), 537–549.

(12) Gerngross, O., *Ber.*, 38 (1905), 3399.

(13) Traube, W., *Ibid.*, 26 (1893), 2551–2558.

(14) Michael, A., *J. Prakt. Chem.*, 157 (1894), 26–43.

Condensation of Amino Acids with Terpenes

I. Aminoacetic Acid and Limonene Nitrosochloride*

By C. F. Krewson

This paper is presented as an introduction to a research project in which it is planned to prepare a variety of condensation products of amino acids and terpenes and to study their biological properties. These remarks will be confined to the condensation of aminoacetic acid and limonene nitrosochloride.

The behavior of nitrosochlorides of terpenes toward a variety of bases has been studied and three different types of reactions observed.

I. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + KOH \xrightarrow{-HCl}$

Terpene nitrosochloride (or heat)

$C_{10}H_{15}NO \rightarrow C_{10}H_{14}NOH$

Nitroso-terpene Isonitroso-terpene (oxime), (1)

II. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + 2HNHR' \text{ (or } HNR') \rightarrow$

Terpene nitrosochloride Primary or secondary base

$C_{10}H_{15}\begin{cases}NOH\\NHR'\end{cases} + HNH.HCl.R'$

Nitrolamine Hydrochloride of base

III. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} \xrightarrow{-NOCl} C_{10}H_{16}$

Terpene nitrosochloride Regenerated terpene, (2)

Reactions of type II have long been used to characterize terpenes since nitrolamines of this type are well-defined crystalline compounds.

Several preliminary experiments with aminoacetic acid (2 moles) and limonene nitrosochloride (1 mole) indicated that these compounds do not combine quantitatively according to reaction II but that the former seems to behave like an inorganic base removing hydrogen chloride, a fact verified by the isolation of carvoxime and the recovery of much of the aminoacetic acid as a hydrochloride.

The reaction products obtained from several experiments where equimolecular quantities of reactants were used showed the presence of the compound desired, a new compound, limonene nitrolaminoacetic acid hydrochloride, [Glycine, *N*-(2-oxo-1-$\Delta^{8(9)}$-*p*-menthenyl)-, oxime hydrochloride], and the volatile oil obtained by steam distillation of the reaction products was found to contain, not only carvoxime, but also a relatively large amount of carvone apparently a result of the hydrolysis of carvoxime to carvone and hydroxylamine.

EXPERIMENTAL

In order to effect condensation 60 Gm. of aminoacetic acid and 158 Gm. of limonene nitrosochloride,

* Presented before the Division of Medicinal Chemistry at the ninety-ninth meeting of the American Chemical Society, Cincinnati, Ohio, April 11, 1940, as a contribution from the laboratory of Edward Kremers, University of Wisconsin, and the Department of Chemistry, University of Kentucky.

dissolved in 600 cc. of 85 per cent alcohol, were warmed to 50° C. for several hours and the mixture distilled with steam to remove volatile products. The oil obtained by the extraction of the distillate with ether amounted to 80 Gm., 36.7 per cent of the original weight of the reacting materials. Spontaneous evaporation of the water distillate left no residue.

A quantitative estimation of the amount of aldehydes and ketones in the volatile oil by use of the Burgess modification (3) of Tiemann's sulfite method gave a value of 41.7 per cent by weight. Rough separation of the oil into two main fractions was secured by use of an eight-inch Vigreux column with reduced pressure. The fraction of lower boiling range amounted to 34 Gm., 42.5 per cent of the volatile oil by weight, a figure in close agreement with that reported for the per cent aldehydes and ketones. This fraction, after purification by two redistillations, one at atmospheric pressure using an ordinary distilling flask, and one under reduced pressure using a ten-plate Podbielniak column [see (4) for column specifications], gave a product having a constant index of refraction, n_D^{20} 1.4960, which was identified as carvone. The yield of carvone was 28.9 per cent of that theoretically possible.

The fraction of higher boiling range obtained from the Vigreux column distillation weighing 25.9 Gm. solidified, and the crystalline mass, which was filtered with the aid of suction, weighed 12.5 Gm., *i. e.*, 15.6 per cent of the volatile oil. These crystals, amounting to 9.7 per cent of the theoretical yield, were characterized as carvoxime and their oil-filtrate failed to show reactions characteristic of carvone, carvoxime or carvacrol, but gave positive qualitative tests for nitrogen and for chloride after fusion with sodium.

An attempt was made to fractionate this oil-filtrate by use of an 18-inch spinning-band column of the Lesesne and Lochte type (5) having a plate value of 19.7 (4). The variation of indices, n_D^{20} 1.4920–1.5210, shown by the thirteen fractions distilled is evidence of the complexity of the material examined. At least two compounds, n_D^{20} 1.5205, n_D^{20} 1.5066, were present in the first nine fractions, these having been obtained before any decomposition became apparent in the column. Each fraction was non-nitrogenous, gave no test for chloride and did not react with phenylhydrazine. Marked decomposition occurred between the take-off of the ninth and tenth fractions and four more fractions were taken after the column cleared but the indices of these fractions showed no sharp breaks, grading from n_D^{20} 1.5090–1.5192. These fractions were found to contain the nitrogenous and the chloride portion of the original oil-filtrate. The limited amount of material curtailed thorough examination of the oil-filtrate.

From the water portion in the flask after the steam distillation of the volatile oil, 39.9 per cent of the aminoacetic acid used was recovered as aminoacetic acid hydrochloride and 3.1 per cent accounted for as a new compound, Glycine, *N*-(2-oxo-1-$\Delta^{8(9)}$-*p*-menthenyl)-, oxime hydrochloride. The residual tarry matter obtained from the steam distillation indicated that considerable polymerization had taken place and it was discarded. The Glycine, *N*-(2-oxo-1-$\Delta^{8(9)}$-*p*-menthenyl)-, oxime hydrochloride, after several recrystallizations from water-alcohol, gave a melting point of 141.0–141.5° C. (uncorrected) and was found to be very soluble in water, less soluble in alcohol and only slightly soluble in ether. Upon analysis for nitrogen using the Kjeldahl method, a 202.5-mg. sample gave a value of 10.14 per cent and a 204.0-mg. sample gave a value of 10.39 per cent, values in close agreement with that of 10.14 per cent, the theoretical amount for the compound $C_{10}H_{15}\begin{cases}NOH\\(NH.HCl)CH_2COOH\end{cases}$. A 312.0-mg. sample of this compound gave 70.8 mg. of copper oxide equivalent to 18.12 per cent copper and a 344.6-mg. sample gave 76.2 mg. of copper oxide equivalent to 17.66 per cent copper, values in agreement with the theoretical 16.95 per cent in the compound, $Cu\left(C_{10}H_{15}\begin{cases}NOH\\NHCH_2COO\end{cases}\right)_2.CuCl_2.2H_2O$, a compound similar to $Cu(A')_2.Cu(NO_3)_2.2H_2O$ which has been reported in the literature (6).

CONCLUSIONS

The reaction between aminoacetic acid and limonene nitrosochloride has been investigated and the following equations are offered to explain the formation of the principal reaction products identified:

I. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + CH_2NH_2COOH \rightarrow$

Limonene nitrosochloride — Aminoacetic acid

$C_{10}H_{14}NOH + CH_2(NH_2.HCl)COOH$

Carvoxime — Aminoacetic acid hydrochloride

II. $C_{10}H_{14}NOH + H_2O \rightarrow$

$C_{10}H_{14}O + NH_2OH$

Carvone — Hydroxylamine

III. $C_{10}H_{16}\begin{cases}NO\\Cl\end{cases} + CH_2NH_2COOH \rightarrow$

$C_{10}H_{15}\begin{cases}NOH\\(NH.HCl)CH_2COOH\end{cases}$

Glycine, *N*-(2-oxo-1-$\Delta^{8(9)}$-*p*-menthenyl)-, oxime hydrochloride

The major portion of the limonene nitrosochloride is used to bring about the first two steps, only a small amount undergoing condensation with aminoacetic acid. No regenerated limonene could be found although considerable tarry residue was obtained during steam distillation of the reaction products.

BIBLIOGRAPHY

(1) Tilden, W. A., and Shenstone, W. A., *J. Chem. Soc.*, 31 (1877), 554; Goldschmidt, H., and Zürrer, R., *Ber.*, 18 (1885), 1729, 2223; Urban, L. C., and Kremers, Ed., *Am. Chem. J.*, 16 (1894), 404; Schmidt Julius, *Ber.*, 35 (1902), 2336.

(2) Wallach, Otto, *Ann.*, 252 (1889), 132; *Ibid.*, 258 (1890), 343.

(3) Gildemeister, E., and Hoffmann, F., "The Volatile Oils," John Wiley and Sons, New York (1913), Vol. I, page 585.

(4) Baker, R. H., Barkenbus, Chas., and Roswell, R. A., *Ind. Eng. Chem., Anal. Ed.*, 12 (1940), 468.

(5) Lesesne, S. D., and Lochte, H. L., *Ind. Eng. Chem., Anal. Ed.*, 10 (1938), 450.

(6) Muir, M. M. P., and Morley, H. F., "Watts' Dictionary of Chemistry," Longmans, Green and Co., New York (1919), Vol. II, page 628.

The Qualitative Detection of Methanol*

By Walter C. Gakenheimer† and Walter H. Hartung‡

An attempt was made to adapt, as a general laboratory exercise, the qualitative U. S. P. test for methanol in whisky (1) as a general method for detecting methyl alcohol in the presence of ethyl alcohol. In the class assignment, every student reported a positive reaction when no methanol was present. This is not surprising when it is realized that fuchsin-sulfurous acid T.S. is a general reagent for identifying aldehydes as a class (2). Under the U. S. P. conditions of the test, it is obvious that ethanol must be oxidized, at least in part, to acetaldehyde.

Eegriwe has reported (3) that chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid) is a sensitive, specific and characteristic reagent for formaldehyde. His procedure has been adopted by Feigl (4) to apply to methyl alcohol. In Feigl's discussion, he says that "with chromotropic acid in a concentrated sulfuric acid solution, a violet-red color appears" and "the following give no reaction: acetaldehyde, propionic aldehyde, butyric aldehyde, isobutyric aldehyde, isovaleric aldehyde, oenanthol, crotonaldehyde, chloral hydrate, glyoxal and aromatic aldehydes. Glyceryl aldehyde, furfural, arabinose, fructose and sucrose give yellow colors. Other sugars, acetone and carboxylic acids do not react. High concentrations of furfural give a red color."

Accordingly, several series of experiments were designed to show:

(*a*) the sensitivity of fuchsin-sulfurous acid T.S. to acetaldehyde;

(*b*) the sensitivity of fuchsin-sulfurous acid T.S. to ethanol (after oxidation according to the U. S. P. procedure);

(*c*) the sensitivity of chromotropic acid to methanol in ethanol;

(*d*) the sensitivity of chromotropic acid to methanol in whisky.

EXPERIMENTAL

Fuchsin-Sulfurous Acid T.S. and Acetaldehyde.— Five cc. of fuchsin-sulfurous acid T.S. was added to 10 cc. quantities of aqueous acetaldehyde solutions of the following concentrations: 1%, 0.1%, 0.01% and 0.001%. A positive reaction was obtained with concentrations of 0.01% or higher, and negative results with the 0.001% solution, showing a sensitivity of at least 1:10,000.

Fuchsin-Sulfurous Acid T.S. and Ethanol.— The U. S. P. procedure for detecting methanol was applied to aqueous solutions of ethanol varying in concentration from 50% to 0.01% and in every case a positive reaction was obtained. By using a blank, it was determined that the oxalic acid-sulfuric acid solution of the Pharmacopœia was sufficient in itself to restore the color to the fuchsin-sulfurous acid T.S.

Chromotropic Acid and Methanol in Ethanol.— The above facts indicate the necessity for an improved test for the presence of methyl alcohol in ethyl alcohol. Consequently, the procedure of Eegriwe, as outlined by Feigl, was applied to a series of solutions of methanol in 50% (by volume) ethanol. The concentrations of methanol used were: 1%, 0.75%, 0.50%, 0.25%, 0.10%, 0.075%, 0.050%, 0.025% and 0.01%. All concentrations as low as 0.1% gave a positive reaction to the naked eye. Those of 0.075% and less gave a negative reaction. It is noteworthy that this test is carried out with 0.02 cc. of the sample and is sensitive to 16γ (0.016 milligram) of methanol.

The procedure consists in mixing drop quantities of 5% phosphoric acid, 5% potassium permanganate solution and the sample. The mixture is allowed to stand a minute and is then decolorized with a little solid sodium bisulfite, after which 4 cc. of 72% sulfuric acid and a little finely powdered chromotropic acid are added. The mixture is well shaken and heated to 60° for 10 minutes. A violet

* From the Department of Chemistry, School of Pharmacy, University of Maryland.
† Alumni Fellow, 1939–1940.
‡ Professor of Pharmaceutical Chemistry.

color, intensifying on cooling, indicates the presence of methyl alcohol.

This test is as simple to carry out as is the U. S. P. method using fuchsin-sulfurous acid T.S. and, in addition, eliminates the need for freshly prepared solutions. Furthermore, chromotropic acid is readily available and at a price which makes the cost per test almost negligible.

Chromotropic Acid and Methanol in Whisky.—In order to determine whether whisky contains interfering substances, the tests above were repeated, using whisky in the place of 50% ethanol. The same order of sensitivity was observed, again detecting 0.016 milligrams of methanol. Pure whisky gave negative results.

CONCLUSIONS

1. The U. S. P. test for methanol in whisky gives a positive reaction with ethanol even though no methanol is present.

2. Chromotropic acid is specific and extremely sensitive to formaldehyde and may be employed in detecting methanol in amounts as little as 0.016 milligrams. Ethanol and whisky do not interfere with this test.

3. The chromotropic acid method is dependable as a specific test for methanol, it is easily carried out and it may be applied to samples as small as 0.02 cc. of a 1:1000 solution of methanol.

REFERENCES

(1) Pharmacopœia of the United States of America. Eleventh revision, page 355.

(2) Shriner and Fuson, "The Systematic Identification of Organic Compounds," (Second edition), John Wiley and Sons, New York (1940), page 62.

(3) Eegriwe, *Z., anal. Chem.*, 110 (1937), 22–25; through *Chem. Abstr.*, 31 (1937), 8442.

(4) Feigl, "Qualitative Analysis by Spot Tests." Translated by Matthews, Nordemann Publishing Company, New York, (1939), pages 327–329.

The following scientists have been honored by France by portrayal on postage stamps: Marcelin Berthelot, pioneer in organic synthesis; Claude Bernard, physiologist; Louis Pasteur, Pierre Curie and Marie Curie, discoverers of polonium and radium; Leon Calmette, research worker in tuberculosis.

Determination of Unsaturates in Cyclopropane*

By Frederick K. Bell and John C. Krantz, Jr.†

The quantitative determination of the amount of unsaturated hydrocarbons present in cyclopropane as impurity presents an interesting and difficult problem. In the preparation of cyclopropane the formation of some unsaturated hydrocarbons is to be expected especially since the chemical procedure involves the transition from a straight chain to a closed ring structure.

There is evidence to indicate that certain of these unsaturated compounds predispose the patient to pulmonary edema, therefore, it is important both to the manufacturer and to the patient that suitable methods be available for determining the efficiency of purification methods employed before this anesthetic gas is used.

Cyclopropane or trimethylene, is the simplest possible cyclic hydrocarbon and it is the least stable of the cyclic hydrocarbons, the stability increasing markedly in passing to the tetra or the pentamethylene ring. It is not surprising then that cyclopropane displays in its chemical reactivity many of the properties ascribed to straight chain unsaturated hydrocarbons, for with simple rupture of the cyclopropane ring the unsaturated hydrocarbon, propene, results.

There are three unsaturated hydrocarbons which are most likely to occur in the preparation of cyclopropane: (1) propene, or methylethene, which is the straight chain isomer of cyclopropane; (2) propadiene, C_3H_4, which contains two double bonds and has a boiling point very near to that of cyclopropane; (3) propyne, or methyl acetylene, which is isomeric with propadiene and contains one triple bond. Reactions typical of these compounds such as the ease of hydrogenation and halogenation, oxidation by potassium permanganate and addition of hydriodic acid are all displayed to some extent by cyclopropane and at the present time no specific reaction has been discovered

---

* Presented to the Scientific Section of the A. PH. A., Richmond meeting, 1940.

† Department of Pharmacology, School of Medicine, University of Maryland Baltimore, Maryland.

which can be used for the quantitative separation of cyclopropane and unsaturated hydrocarbons.

The potassium permanganate method was selected and introduced into the U. S. P. monograph for cyclopropane. In this method one liter of the gas to be examined is passed, under closely specified conditions, through 50 cc. of hundredth-normal potassium permanganate which has been cooled and is maintained at approximately 0° C. To be sure, under these conditions the efficiency of the absorption of unsaturates could not be expected to be high, but it was hoped that by using dilute and cold permanganate solution the oxidation of the cyclopropane could be practically eliminated so that the results finally obtained would bear a simple and direct relation to the amount of unsaturates present in the liter gas sample.

After the passage of the gas through the permanganate solution, the latter is promptly transferred to a suitable flask and 50 cc. of hundredth-normal oxalic acid are added. The mixture is heated to 80° C. and then titrated with hundredth-normal potassium permanganate in the presence of sulfuric acid. This titer is a measure of the quantity of unsaturates present and the Pharmacopœia requires that it shall not exceed 10 cc.

EXPERIMENTAL

Owing to time restrictions, an extensive study of the permanganate test could not be completed. The results that were obtained indicated that the method had considerable merit although it was recognized that the reproducibility of results was not entirely satisfactory. We have recently completed a more comprehensive examination of the present pharmacopœial test with and without certain modifications. In this work we have had the coöperation of various manufacturers of cyclopropane through one of whom we also obtained an especially purified sample which we have designated as C.P. Several commercial samples of cyclopropane were examined as well as some special ones showing a high value for unsaturates. Nearly 200 analyses were carried out.

In the following table a summary of the results obtained by the pharmacopœial method are shown. Average values are indicated.

It is seen at once that the reproducibility of results is not only of low order but also appears to vary with the particular gas sample being analyzed and this suggested that at least one important variable of the system was not under control. In this connection we directed our attention to a consideration of the ultimate fate of the cyclopropane dissolved in the cold permanganate solution. After the passage of the liter-gas sample through this cold solution it is probable that nearly 20 cc. of cyclopropane are dissolved therein.

Table I

Sample	Cc. *N*/100 $KMnO_4$ Titer	Deviation
A	4.2	0.36
B	18.5	0.65
C	4.7	0.18
D	4.7	0.34
E (C.P.)	3.0	0.42

In a second series of experiments we have modified the pharmacopœial method in the following manner. The cold permanganate solution through which the cyclopropane sample has been bubbled is added in small portions to a mixture of 50 cc. of hundredth-normal oxalic acid and 5 cc. of sulfuric acid which has been heated to 90° C. After each addition of the permanganate the mixture is shaken for a few seconds until it becomes colorless. After the complete transfer of the permanganate solution the mixture, which then has a temperature of 55 to 60° C., is titrated with hundredth-normal permanganate. The average values obtained by this modified method are as follows:

Table II

Sample	Cc. *N*/100 $KMnO_4$ Titer	Deviation
B	18.4	0.6
C	4.2	0.21
D	3.7	0.37
E (C.P.)	1.8	0.23
F	12.4	0.45

CONCLUSIONS

The results show a slight improvement of reproducibility as shown by a general decrease in the average deviation. In the modified procedure, there is also a general decrease in the magnitude of the permanganate titer which is especially marked in the case of the C.P. cyclopropane (sample E) where the value has dropped from 3 cc. to 1.8 cc. Although the modified procedure does not produce any marked improvement in the accuracy of the method, it is believed to be desirable since it attempts to further standardize the procedure particularly at the point where the source of the difficulty most likely occurs.

Either the pharmacopœial method or the modified form may be regarded as ade-

quate with regard to the present cyclopropane market and methods of production. Any marked change in these factors would undoubtedly call for an immediate review of the method. Therefore, it is clearly indicated that further studies should be undertaken.

Detection of Quinicine and Cinchonicine*

By J. W. Millar and S. J. Dean

The presence of quinicine (quinotoxin) and cinchonicine (cinchotoxin) in preparations containing quinine and cinchonine has frequently been questioned. A number of tests have been proposed, some of which are not satisfactory. This study was undertaken to obtain satisfactory tests for these substances and to determine in each case the minimum concentration in which detection can be made in aqueous and in alcoholic solutions, also when they are present with the unchanged parent alkaloid.

EXPERIMENTAL

Preparation of Cinchonicine and Quinicine.—Employing the method used by Biddle (1), ten grams of cinchonine, ten grams of glacial acetic acid and one hundred and thirty cc. of water were heated to boiling for thirty-five hours. The cooled brownish red solution was treated with an excess of sodium hydroxide and the light brown oil which separated was extracted with ether. The ether solution was dried with calcium carbide. Any unchanged cinchonine, which is insoluble in dry ether, is precipitated when the solution is dried.

The excess of ether was evaporated and the cinchonine dissolved in alcohol or water.

Quinicine was prepared from quinine in a similar manner.

Diazo-Benzene Sulfonic Acid Reagent.—Biddle (2) makes use of this reagent for the identification of cinchonicine and quinicine.

Procedure: two cc. of a freshly prepared saturated aqueous solution of diazobenzene sulfonic acid rendered alkaline with a few drops of sodium hydroxide is added to ten cc. of a solution of quinicine. The color produced varies from a light pink to a reddish violet according to the amount of quinicine present. The color must appear within five minutes. The minimum concentration of quinicine in aqueous solutions yielding distinctive tests was found to be 1 part in 12,500.

Alcoholic solutions reduce the sensitivity of the test to 1 part in 2500.

To determine the delicacy of this test in the presence of soluble salts of quinine, saturated solutions of the hydrobromide, hydrochloride and sulfate containing known amounts of quinicine were used.

The minimum concentration of quinicine yielding satisfactory tests was 1 part in 6250.

Tests performed with cinchonicine and diazobenzene sulfonic acid in the same manner in aqueous solution, yielded distinctive tests in dilutions of 1 part in 6250.

In alcoholic solution, the sensitivity was 1 part in 3000.

With saturated solutions of salts of cinchonine, the sensitivity of the test was 1 part in 3500.

Dinitro Thiophene Reagent.—Biddle (3) also made use of this reagent for the identification of quinicine and cinchonicine.

It is prepared by dissolving dinitro thiophene in pure nitro benzene (1:200).

To two cc. of the reagent is added a few drops of alcohol and then the alcoholic or ethereal solution of the substance to be tested.

The color produced varies from a light orange to a deep purple-red according to the amount of toxin present. The solution tested must be neutral and the test is unreliable in the presence of salts of quinine or cinchonine.

The minimum concentration of quinicine yielding distinctive tests was 1 part in 2700. In the presence of quinine alkaloid the sensitivity was 1 part in 480.

With cinchonicine, tested in the same manner, the minimum concentration yielding distinctive tests was 1 part in 1740, and in the presence of cinchonine alkaloid the sensitivity was 1 part in 600.

Lipkin's Test.—Lipkin (4) describes the following test to differentiate between quinine and quinicine:

"To 5 cc. of the solution to be tested is added 2–3 drops of a 0.5% aqueous solution of Congo Red. Bromine water is added until a yellow color is obtained; ammonia added immediately and the solution extracted with chloroform. Quinine yields a green color in the chloroform extract and quinicine a red color."

Tests were made replacing Congo Red in the above test with other indicators, *viz.*, brom-phenol blue, brom-cresol green, methyl red, thymol blue, phenol red, brom-thymol blue, brom-cresol purple, and with each indicator the resulting color was the same for quinicine and no color change for cinchonicine.

The same test was made on quinicine and cinchonicine omitting the indicator; the same results were obtained.

It is suggested that this test (no indicator being used) may be used for the differentiation of quinicine and cinchonicine.

---

* A contribution from the laboratories of the College of Pharmacy, University of California, Medical Center, San Francisco, California.

Ball's Test.—Ball (5) proposed the use of potassium ferrocyanide as a reagent for cinchonine.

The test is described as follows: "Potassium ferrocyanide if added to the solution of a salt of this alkaloid, produces a yellowish white curdy precipitate, which is dissolved upon the application of a gentle heat, but is again deposited, when the liquid cools, as an abundant crop of golden yellow crystals; a slight excess of ferrocyanide should be used."

This test, with some modifications was used on quinicine and cinchonicine. The test as used was: to an aqueous solution of the toxin, add an equal volume of dilute sulfuric acid (6*N*) and then an excess of a solution of potassium ferrocyanide.

Quinicine yields a brilliant yellow precipitate not dissolved by boiling.

Cinchonicine yields a salmon pink precipitate not dissolved by boiling.

Sensitivity for both toxins 1:60.

The phenylhydrazine and nitrous acid tests of Ganassini (6) were tried and discarded as unsatisfactory.

SUMMARY

1. Diazobenzene sulfonic acid reagent gives reliable tests for quinicine and cinchonicine in aqueous or alcoholic solutions and in the presence of the parent alkaloid or alkaloidal salts.

2. Dinitro thiophene reagent gives reliable tests for quinicine and cinchonicine in alcoholic or ethereal solution and in the presence of the parent alkaloid, but not in the presence of the alkaloidal salts.

3. The modified Lipkin test may be used to differentiate between quinine and quinicine and between cinchonine and cinchonicine.

4. The modified Ball test may also be used to differentiate between quinicine and cinchonicine.

5. The phenylhydrazine and nitrous acid tests are less satisfactory than others described.

REFERENCES

(1) *Journ. A. C. S.*, 34 (1912), 502.
(2) *Ibid.*, 34 (1912), 507.
(3) *Ibid.*, 34 (1912), 508.
(4) Lipkin, I. J., *Chem. Abstr.*, 14 (1920), 1580.
(5) Ball, J. W., U. S. Dispensatory, 21st Edition (1926), page 347.
(6) Ganassini, D., *Chem. Abstr.*, 16 (1922), 2008.

"Clearness is the ornament of profound thought"—Vauvenargues

The Stabilizing Effects of Antioxidants upon Solution of Tannic Acid*

By K. P. DuBois† and C. O. Lee‡

The decomposition of tannic acid solutions is believed to be due, in part at least, to oxidation. This belief suggested the possibility of using antioxidants as protective agents for such solutions. As a result of our study we believe that very satisfactory stabilizing agents have been found for tannic acid solutions.

In so far as we were able, we applied the information which had been obtained in various other antioxidant studies to the problem of stabilizing tannin solutions. It was realized that an antioxidant must be very active in order to be effective in stabilizing tannin solutions because they themselves act as very active antioxidants under certain conditions.

EXPERIMENTAL

Assay Method for Tannin Solutions.—The effectiveness of the various antioxidants is dependent upon their ability to prevent the loss of tannin in tannic acid solutions. This makes it necessary to determine the tannin content of such solutions at frequent intervals. A modification of the Lowenthal-Proctor method was found to be satisfactory, especially so with simple solutions of tannic acid (1).

The following tannic acid solution has been recommended for therapeutic use (2). This solution without the salicylic acid was made the subject of our stabilization study.

Tannic acid	100.00 Gm.
Sodium chloride	10.50 Gm.
Potassium chloride	0.42 Gm.
Calcium chloride	0.84 Gm.
Salicyclic acid	1.00 Gm.
Distilled water to make	1000.00 cc.

One hundred cubic centimeter portions of this solution were placed in 120-cc. prescription bottles. To each of 17 of them a different antioxidant was added. In addition one sample was stored under carbon dioxide, another under nitrogen to exclude the air, and a third served as the control.

Effectiveness of Common Antioxidants.—The antioxidants used are listed in Table I. Most of these

---

* An abstract of a Thesis presented to the faculty of Purdue University in partial fulfilment of the requirements for the degree of Master of Science.

† AMERICAN PHARMACEUTICAL ASSOCIATION Fellow, 1939–1940.

‡ Professor of Pharmacy, Purdue University.

substances have been known to behave as antioxidants or are thought to possess such property because of their ease of oxidation. The antioxidants used in this series were representative compounds selected from each of the various classes of chemical antioxidants.

Table I.—Effectiveness of the More Common Antioxidants

Sample No.	Antioxidant	Per Cent Tannin			Per Cent Loss of Tannin	
		Immediately after Preparation	After 60 Days	After 120 Days	After 60 Days	After 120 Days
16	Sodium sulfite	9.35	9.35	9.35	0	0
18	Sodium thiosulfate	9.35	9.37	9.35	0	0
15	Sodium sulfite (1%)	9.35	9.28	9.26	0.7	0.96
19	Carbon dioxide	9.35	9.18	9.02	1.81	3.53
17	Phosphoric acid	9.35	9.00	8.90	3.94	4.81
5	Tartaric acid	9.35	9.08	8.90	2.78	4.81
8	Resorcinol	9.35	8.95	8.75	4.81	6.41
7	Hydroquinone	9.35	8.95	8.75	5.33	6.41
12	Sodium citrate	9.35	9.04	8.69	3.30	7.16
2	Salicylic acid	9.35	8.85	8.69	5.33	7.16
3	Benzoic acid	9.35	8.85	8.69	5.33	7.16
20	Nitrogen	9.35	8.85	8.61	5.33	7.91
10	Allyl alcohol	9.35	8.85	8.52	5.33	8.87
4	Citric acid	9.35	8.61	8.52	7.91	8.87
1	No antioxidant	9.35	8.64	8.44	7.59	9.73
11	Benzyl benzoate	9.35	8.52	8.38	8.87	10.38
14	Zinc chloride	9.35	8.84	7.95	5.36	15.90
13	Methenamine	9.35	8.64	7.81	7.59	16.47
9	Glycine	9.35	8.78	7.70	6.01	17.64
6	Pyrogallic acid	9.35	8.28	5.02	11.33	46.31

Table II.—Effectiveness of Less Common Oxidants

Sample No.	Antioxidant	Concentration of Antioxidant	Per Cent Tannin		Per Cent Loss of Tannin
			Immediately after Preparation	After 60 Days	After 60 Days
35	Maleic acid	0.2	8.58	8.20	4.43
32	Anisol	0.2	8.58	8.20	4.43
33	Chloroform	0.2	8.58	8.20	4.43
34	Parahydroxybenzaldehyde	0.2	8.58	8.20	4.43
38	Malonic acid	0.2	8.58	8.20	4.43
39	Gum guaiac	0.2	8.58	8.20	4.43
40	Soy bean oil	0.2	8.58	8.20	4.43
42	Tincture of Merthiolate	0.2	8.58	8.20	4.43
43	Salicylic acid	0.2	8.58	8.20	4.43
41	Glycerin	1.0	8.58	8.12	5.36
22	Control	...	9.32	8.80	5.58
36	Cholesterol	0.2	8.58	8.03	6.40
37	Aniline	0.2	8.58	8.03	6.40
24	Fructose	0.2	9.32	8.72	6.44
27	Sodium hypophosphite	0.2	9.32	8.46	9.22
26	*l*-Arabinose	0.2	9.32	8.37	10.19
23	Rhamnose	0.2	9.32	7.21	22.64
25	*l*-Xylose	0.2	9.32	6.94	25.53

The concentration of the antioxidants was 0.2 per cent in all samples except those stored under the gases and sample 15 to which 1.0 per cent of sodium sulfite was added 24 hours after being prepared.

All samples were assayed for tannin content immediately after being prepared. The bottles were then sealed with paraffin and stored in a dark place. Assays were again made after 60 days and 120 days. The results of the assays are shown in the table arranged in the order of the increasing loss of tannin after 120 days.

Observations and Comments.—The addition of 0.2% sodium sulfite prevented the characteristic loss of tannin and the usual discoloration of the preparation. The addition of the same concentration of sodium thiosulfate produced a similar effect, except that a small amount of free sulfur was deposited in the container. Storing under carbon dioxide gas partially prevented tannin loss and discoloration. Nitrogen was also partially effective. It is believed that absolute exclusion and displacement of oxygen from these samples was not obtained. The addition of sodium sulfite 24 hours after preparation of the sample 15 did not give full protection against decomposition. This seems to indicate that the deterioration begins immediately after preparation of the samples and that the antioxidants should be added during preparation of the solution.

It was noted that in all samples decrease in tannin content was accompanied by increase in discoloration. Hydroquinone, although it has been used as a very effective antioxidant in other stabilization studies, was of no particular value in this experi-

ment. This bears out the fact that the effectiveness of an antioxidant is dependent upon the nature of the compound which is to be stabilized. Other substances used in this series showed no appreciable stabilizing effect, some of them even hastening decomposition. These substances were eliminated from further consideration of this problem.

Addition of Less Common Antioxidants to Tannic Acid Solutions.—Caldwell and Bibbins (3) in their work on antioxidants concluded that the "trial and error" method must be used to determine the possible effectiveness of an antioxidant and the amount necessary to stabilize the substance under study. Sugars have unexpectedly shown antioxidant properties. Amines also are known to have antioxygenic properties (4).

For a second study some less commonly used antioxidants such as aniline, maleic acid, anisol and some sugars were added to a series of tannic acid solutions. These were stored in prescription bottles and protected from light. Tannic acid determinations were made when prepared and at the end of sixty days. The results are given in Table II.

Observations and Comments.—None of the antioxygenic substances used in this series prevented tannin loss. The sugars apparently hastened decomposition and provided a media for mold growth. Tincture of Merthiolate, although not an antioxidant, hindered mold growth but did not prevent discoloration of the solutions or loss of tannin. Anisol, chloroform, *p*-hydroxybenzaldehyde, aniline, soy bean oil, malonic acid and gum guaiac were not effective stabilizing agents and were eliminated from further study.

Comparison of Sulfite Compounds as Stabilizing Agents.—Since sodium sulfite and sodium thiosulfate had previously appeared to be the only good antioxidants for the preservation of tannic acid solutions a number of more detailed experiments were carried out with sulfite compounds. It would seem logical to expect all sulfite salts to act in a manner similar to sodium sulfite. However, in order to obtain experimental evidence regarding the action of these compounds, comparisons between sodium sulfite, sodium thiosulfate, potassium sulfite and sodium bisulfite were made. The substances were added in concentrations of 0.2% to the tannic acid solution previously described. After standing protected from light for 60 days tannin determinations were made, the results of which are given in Table III.

Observations and Comments.—Each of these antioxidants prevented tannin loss and discoloration of the solutions. There was light decomposition of the sample containing sodium thiosulfate, as well as liberation of free sulfur.

Minimum Effective Concentration of Sodium Sulfite.—In order to determine the minimum concentration of sodium sulfite which would completely prevent tannin loss various concentrations of it were added to a number of tannic acid solutions. Concentrations of sodium sulfite ranging from 0.01 to 2.0% were used in this experiment. The results obtained on tannin determinations appear in Table IV.

Table IV.—Minimum Effective Concentration of Sodium Sulfite

Sample No.	Concentration of Sodium Sulfite	Per Cent Tannin Immediately after Preparation	Per Cent Tannin After 60 Days	Per Cent Loss of Tannin After 60 Days
58	Control	8.58	8.03	6.40
45	0.01%	8.58	8.03	6.40
46	0.03	8.58	8.12	5.36
47	0.05	8.58	8.33	2.91
48	0.07	8.58	8.49	1.04
49	0.10	8.58	8.56	0
50	0.20	8.58	8.59	0
51	0.30	8.58	8.59	0
52	0.40	8.58	8.59	0
53	0.50	8.58	8.59	0
54	0.70	8.58	8.64	0
55	1.00	8.58	8.59	0
56	1.50	8.58	8.64	0
57	2.00	8.58	8.59	0

Observations and Comments.—The minimum concentration of sodium sulfite required to prevent tannin loss was 0.1%. Concentrations of the antioxidant below 0.1% were only partially effective, the 0.01% being ineffective. All concentrations above 0.1% were effective. The solutions containing 1.5 and 2.0% contained a portion of undissolved sodium sulfite.

The prevention of tannin loss and discoloration of tannic acid solutions by sodium sulfite suggests its use in the preparations of tannic acid solutions to be used medicinally. Sollmann (5) states that sulfites are not active therapeutically in low concentrations, thus they may be used without fear of producing untoward physiological effects.

The use of antioxidants as protective agents for tannin solutions presents the probability that atmospheric oxygen plays a part in the decomposition. This was substantiated by Lefevre and Lee

Table III.—Comparison of Effectiveness of Sulfite Compounds

Sample No.	Antioxidant	Concentration of Antioxidant	Per Cent Tannin Immediately after Preparation	Per Cent Tannin After 60 Days	Per Cent Loss of Tannin After 60 Days
21	Control	...	9.32	8.80	5.58
28	Sodium sulfite	0.2	9.32	9.35	0
29	Sodium bisulfite	0.2	9.32	9.35	0
30	Sodium thiosulfate	0.2	9.32	9.26	0.6
31	Potassium sulfite	0.2	9.32	9.35	0

(6) who stored Fluidextracts of Krameria for 12 months in air-tight containers and compared the loss of tannin with the loss observed in the same samples which had not been sealed. It was found that the tannin loss in unsealed samples was much greater than that in sealed samples. This further indicates that oxidation plays a part in the decomposition of tannic acid solutions.

SUMMARY AND CONCLUSIONS

1. Results obtained from this study seem to indicate that most of the well-known chemical antioxidants are ineffective in the stabilization of tannic acid solutions.

2. Sodium sulfite and other sulfite salts are effective in preventing discoloration and loss of tannin in aqueous solutions of tannic acid, at least for a period of four months.

3. In aqueous tannic acid solutions decrease in the tannic acid content is not only immediate but accompanied by marked discoloration of the solutions.

4. There is evidence that the presence of atmospheric oxygen contributes to the decomposition of tannic acid solutions.

REFERENCES

(1) A. O. A. C. Methods of Analysis (1930), 155.

(2) Fantus, B., and Dyniewicz, H. A., *Jour. A. M. A.*, 109 (1937), 200.

(3) Caldwell, A. L., and Bibbins, F. E., JOUR. A. PH. A., 23 (1934), 7.

(4) Moureu, C., Dufraisse, C., and Badoche, M., *Compt. rend.*, 183 (1926), 408.

(5) Sollmann, T., "A Manual of Pharmacology" (Fifth edition) (1937), 182.

(6) Lefevre, H. F., and Lee, C. O., Purdue University, 1939.

Homogeneity of Tablet Mixtures Before Granulating*

By E. C. Beeler and E. N. Gathercoal†

Tablet forms have been recognized for many years as one of the most convenient methods of administering medicines with uniform and exact dosage. There have been many phases of tablet making reported in the literature, but few of them have considered the homogeneity of tablet mixtures. The fabrication of compressed tablets consisting of multi-component substances can be divided into four factors wherein uniformity is either consummated or modified; named in the order of processing tablets, they are (1) mixing before granulating, (2) granulating, (3) mixing after granulating prior to compression and (4) compression. The first three factors involve the mixing of relatively small solid particles to uniformity, while the fourth, compression, involves the effect of mechanical vibrations of the tablet machine; any mechanical disturbance in the latter could break the homogeneity established in the granulation.

Any type of mixture, *i. e.*, liquid solution, solid solution, gases, mechanical mixtures, etc., is homogeneous when each component substance possesses a constant proportional relationship to every other component substance in any fractional part of the mixture. An ideal mixture is one in which intermingling of particles of the component substances down to molecular magnitude can be accomplished. A solid substance reduced to powder form by mechanical means could never be made to reach such a minute size; therefore, medical powder mixtures are classified as mechanical mixtures in which homogeneity can never be completely realized. Snow and Fantus (1) advanced the theory on tablet making that the material to be compressed must be heterogeneous with respect to the electrical potential set up by mechanical friction of the particles being compressed.

Homogeneity will be used in this paper to indicate the uniformity of composition to which a tablet mixture can be prepared under specific physical conditions of the component substances. Experimental evidence of homogeneity is obtained from the results of several analyses on at least one of the component substances. Consequently, the term homogeneity as applied to a powder mixture is a general description of the uniformity of composition in a fractional part of the mixture convenient for ordinary analysis.

Conditions entering into the problem of mixing solids with solids are of a mechanical or physical nature. Physical, or inherent, characteristics of the component substances comprise the factors which cannot be controlled while mechanical factors can be

* Presented to the Scientific Section of the A. PH. A., Richmond meeting, 1940.

† From the Laboratory of the AMERICAN PHARMACEUTICAL ASSOCIATION, Washington, D. C.

regulated to a reasonable extent. Some of the physical characteristics are density, electrostatic dynamics, segregation and characteristic surface. Mechanical conditions include particle size and shape, bulkiness (partially controlled by particle size), proportional relationship of ingredients, methods of mixing and rate of mixing.

EXPERIMENTAL

Magnesium pyrophosphate, barium sulfate, sodium carbonate and corn starch were selected for the study of the effects of particle size and particle density of component substances on the homogeneity of powder mixtures. Simple binary mixtures of magnesium pyrophosphate-starch and of barium sulfate-starch were first studied. N. B. S. sieves were used in grading the particle sizes with the exception of starch, used as received, the particle size of which was microscopically determined to range between 5 and 35 microns. Forty-mesh particles refer to all material passing through No. 40 sieve and retained on No. 60. Two-hundred mesh particles refer to those which passed through No. 200 sieve.

Forty-mesh barium sulfate was prepared by completely moistening the finely powdered material with water, drying, igniting and grinding the hard mass to the desired particle size. Its density was determined to be 4.2 Gm. per cc. at 25° C. Two hundred-mesh barium sulfate was sieved from the fine powder before moistening.

Magnesium pyrophosphate was prepared by ignition of ammonium magnesium pyrophosphate, precipitated from magnesium sulfate solution with ammoniacal sodium phosphate solution. The hardened mass was ground to the desired particle size. Forty-mesh magnesium pyrophosphate was found to have a density of 2.3 Gm. per cc. at 25°.

Forty-mesh starch was prepared from oven dried starch paste. The density of packaged starch was determined to be 1.5 Gm. per cc. at 25° C.

The component substances were mixed together by the principle of tumbling by rotation on a diagonal axis of a cylinder. The components to be mixed were placed in a wide-mouth, cylindrical bottle, 7 inches long by $3^1/_2$ inches in diameter, made of clear glass. The bottle was tied in the holders of a horizontal ball mill one quart size, at such an angle that the axis of rotation was approximately on a diagonal of the cylinder. An arrangement was made to tap the bottle at the shoulder at each revolution of the bottle. The bottle was rotated for a period of 12 to 15 hours at a rate of about 40 r. p. m.

After the mixing period, six samples were taken from the top of the mixture without removing the bottle from the ball mill. Sample number 1 in the table of results was taken next to the mouth of the container while number 6 was taken at the rear; samples numbered 2, 3, 4 and 5 were taken in consecutive points between numbers 1 and 6. Approximately 2-Gm. samples were taken, which represented about one-three-hundredth of the total mixture. Inorganic salts in the samples were determined by ignition of the starch. Results of the binary mixtures were as follows:

Table I.—Per Cent of Inorganic Salt in the Mixture

Sample Number	No. 1 No. 200 $BaSO_4$ 5–35 μ Starch Decld.	Found	Effcy.	No. 2 No. 40 $BaSO_4$ 5–35 μ Starch Decld.	Found	Effcy.	No. 3 No. 200 $BaSO_4$ No. 40 μ Starch Decld.	Found	Effcy.
1	19.85	19.72	99.35	20.00	19.88	99.40	20.17	16.72	82.90
2	19.85	19.73	99.45	20.00	20.07	100.35	20.17	16.81	83.34
3	19.85	19.69	99.19	20.00	19.99	99.95	20.17	16.62	82.40
4	19.85	19.44	97.93	20.00	19.96	99.80	20.17	16.90	83.79
5	19.85	19.68	99.14	20.00	19.98	99.90	20.17	16.81	83.34
6	19.85	20.20	101.76	20.00	19.98	99.90	20.17	16.80	83.29
Average			99.47			99.88			83.17
Range of efficiency			3.83			0.95			1.39

Sample Number	No. 4 No. 200 $Mg_2P_2O_7$ 5–35 μ Starch Decld.	Found	Effcy.	No. 5 No. 40 $Mg_2P_2O_7$ 5–35 μ Starch Decld.	Found	Effcy.	No. 6 No. 200 $Mg_2P_2O_7$ No. 40 μ Starch Decld.	Found	Effcy.
1	21.39	22.18	103.69	20.31	20.99	103.35	16.03	15.77	98.38
2	21.39	22.15	103.55	20.31	20.68	101.82	16.03	15.08	94.07
3	21.39	22.15	103.55	20.31	20.41	100.49	16.03	14.91	93.01
4	21.39	22.04	103.04	20.31	18.28	90.00	16.03	14.46	90.21
5	21.39	21.83	102.06	20.31	20.72	102.02	16.03	14.51	90.52
6	21.39	21.84	102.11	20.31	20.73	102.06	16.03	14.87	92.76
Average			103.00			99.95			93.16
Range of efficiency			1.63			13.35			8.17

A ternary powder mixture was prepared using the method described above for mixing the ingredients. The mixture contained the following component substances:

	Weight, Gm.	Per Cent
Corn starch (5–35)	125.13	80.31
Barium sulfate (40-mesh)	24.68	15.84
Reagent sodium carbonate (40-mesh)	6.00	3.85
Total	155.81	100.00

The carbonate was screened from the reagent bottle as received which was almost all 40-mesh.

It was heated to 270° C. for one hour before entering with the other ingredients. The method of mixing and sampling the mixed components were the same as described for the binary mixture.

The assay process was as follows: The weighed sample was placed in a 250-cc. beaker with about 50 cc. of distilled water and stirred to dissolve the carbonate. Insoluble substance was filtered through a Gooch crucible previously ignited and tared, the beaker and residue being washed with several portions of distilled water to complete the transfer. The filtrate was titrated with twentieth-normal sulfuric acid using methyl orange T.S. as indicator; each cc. of the acid was equivalent to 0.0265 Gm. of Na_2CO_3. The residue in the Gooch was ignited and weighed after one hour at 970° C.; the weight of the ignited residue was taken as barium sulfate. Starch was determined by the difference between the total weight of the sample and the sum of the carbonate and sulfate. Each filtrate after titration gave a negative test for starch with iodine T.S. Results of the assays of the ternary mixture were as follows:

Table II.—Results of Assay of Ternary Mixture

Sample No.	Na_2CO_3, 3.85%		$BaSO_4$, 15.84%		Total Inorg., 19.69%		Starch, 81.31%	
	Found	Effcy.	Found	Effcy.	Found	Effcy.	Found	Effcy.
1	3.71	96.36	15.26	96.34	18.97	96.35	81.03	100.90
2	3.78	98.44	15.71	99.18	19.49	98.98	80.01	99.63
3	3.63	94.29	15.04	94.95	18.67	94.82	81.33	101.27
4	3.84	99.74	15.96	100.76	19.80	100.56	80.20	99.00
5	3.81	98.96	15.77	99.56	19.58	99.44	80.42	100.14
6	3.86	100.26	15.80	99.75	19.66	99.85	80.34	100.04
Average		98.00		98.42		98.33		100.13
Range of efficiency		5.97		5.81		5.74		1.64

DISCUSSION OF RESULTS

Mixture No. 2 was found to be closest to theoretical as well as the most uniform of the six binary mixtures, while No. 5 was less uniform and not so close to theoretical. This might be attributed to the difference in physical characteristics of barium sulfate and magnesium pyrophosphate. Magnesium pyrophosphate gave results of efficiency which were higher than with barium sulfate in mixtures with the same particle size of component substances. This fact further substantiates the possibility that the uniformity of a solid-solid mixture depends upon the characteristic surface and density of the component substances.

Forty-mesh barium sulfate mixed uniformly with packaged starch, mixture No. 2, while 200-mesh magnesium pyrophosphate was required to obtain a uniform mixture with starch, mixture No. 4. Forty-mesh starch did not mix well with either of the inorganic salts probably by reason that, being present in the largest amount, it has a considerable volume of air space between particles for the component substance of lesser particle sizes and present in smaller amounts to settle between. All of the samples for analysis were taken from the top of the mixture. It is conceivable that the small particles of inorganic salt were sticking to the larger starch particles and in some places were trapped in the air spaces. Had the amount of inorganic salt been such that it would have filled all the air spaces between the starch particles the mixture might have analyzed closer to theoretical, for there could be no settling of small particles down through the mixture. On the other hand, the packaged starch, being of smaller particle size, fills the air space between particles of 200-mesh or 40-mesh size. The number of small starch particles being greatly in excess of the amount necessary to fill in the spaces between the larger particles, the latter were separated from contact with each other.

Mixture No. 3 was a distinct exception to all the other mixtures. There was a decided tendency by the barium sulfate to adhere to the front and rear of the mixing container during rotation; the particles continued to adhere despite the fact that 0.5% of packaged starch was added to prevent it. This accounts for the deviation of 17 per cent from theoretical in mixture No. 3.

Sample 4 of mixture No. 5 requires some consideration since it is about 10 per cent below theoretical. It is possible that agglomeration of starch particles occurred at this position in the container. Agglomerated particles are likely to be found settling at the point where the least agitation would occur which is about the center of the container somewhere between sample 3 and sample 5. This position is the point where the lowest results were obtained in mixtures having a considerable range of efficiency of mixing.

Results on the ternary mixture, Table II, indicate about 6 per cent range of efficiency in the series of analyses taken at various positions after mixing. A more uniform mixture was expected on the basis of binary mixture No. 2 in Table I and, assuming that the physical characteristics of sodium carbonate simulated those of barium sulfate. The results do stress the importance of physical characteristics of component substance in obtaining the homogeneity of powder mixtures. It is worthy of noting that the minimum of inorganic salts was found between samples 3 and 5 with a maximum in starch content at that point; this is a problem of mixing methods which has not been investigated.

From the results in Tables I and II there is no indication that density of the component substances had any influence upon the homogeneity of the mixtures.

THEORETICAL DISCUSSION

Particle Size, Shape and Density.—Heywood (2) attempts to numerically define particle size and shape, confining the definition to irregular particles, based on a projected image. He was dealing with materials of pebble size so that the projected image was microscopic. However, with small particles the projected image can be obtained with the microscope.

There is evidence (4) that particulate materials obey a normal law of distribution of particle sizes and shapes. Not only does a homogeneous powder mixture (3) conform to a normal distribution law, but its physical state is characterized by surface area per unit volume and particles per unit weight, a quality known as fineness. Roller (4) has shown that size distribution may be determined from the relation between weight per cent and particle size.

Bulkiness is a common expression in referring to a powdered material. It is measured by the amount of air space (voids) contained in a given weight of the material when in packing. Various methods have been given for measuring void content (5, 6), the simplest being to fill a graduated cylinder with a given weight of material and tap it on the bottom until no change in volume is noted.

Void content of a powder mixture is very important in determining the extent of uniform distribution in a mixture of varying particle sizes. There are three essentials necessary in predicting the expected accuracy of dosage and extent of uniformity of a particulate material neglecting surface characteristics (1) the void content, (2) particle size (3) proportion by weight of the active ingredient. Roller (7) has given a simple method for calculating void content in relation to density and specific volume. Circumstance decidedly alters the conditions. In the case of a tablet mixture containing a large amount of excipient material and a small amount of active ingredient the total volume of voids between particles of the excipient should not exceed the volume of the active ingredient, assuming that the ideal condition for homogeneity is that particles of active ingredient are just small enough to fit into the free space surrounded by larger particles of excipient. Therefore, the amount of excipient and the most desirable particle size for the active ingredient that can be used are limited entirely by the particle sizes of the excipient or vice versa. In the case where large amounts of active ingredients contain little excipient, homogeneity would depend entirely upon the extent of uniformity in size of particles of the active ingredient and the amount of excipient that could be used without destroying uniformity would be determined by the void content as in the case above. In the case in between the cases mentioned, *i. e.*, there is about as much ingredient as excipient, the principle of mixing becomes one in which the component substances are put in such a condition that each particle of the active ingredient lies adjacent to a particle of the excipient.

G. F. Smith (8) has shown that, when materials of different density are mixed together followed by grinding to pass a 200-mesh sieve, there is no indication of segregation due to vibrational disturbances. Density is very closely allied to particle size in determining the amounts of excipients necessary for obtaining ideal conditions of mixing the active ingredients. It would seem, therefore, that when component substances are properly mixed there could be no direct variation in accuracy of a tablet mixture due to density of the ingredients.

SUMMARY

From the experimental investigation of powder mixtures carried out in the A. PH. A. Laboratory up to the writing of this report the following have been deduced concerning a tablet mixture before granulating:

1. Physical characteristics of component substances affect the homogeneity of powder mixing.

2. Agglomeration in powder mixtures is intensified as the particle size is reduced. Materials which tend to pack to very small volumes when in very fine powder form show a definite tendency toward agglomeration when mixed with other materials of larger particle size.

3. Particle size and weight per cent is of great importance in determining the amount of excipient to be used.

BIBLIOGRAPHY

(1) Snow, C. M., and Fantus, B., JOUR. A. PH. A., 10 (1921), 850.

(2) Heywood, H., *Chem. Ind.*, 56 (1937), 149.

(3) Roller, P. S., *Am. Soc. Test. Mat.*, 37 (1937), 678–683.

(4) Roller, P. S., *Jour. Fr. Inst.*, 223 (1937), 609–623.

(5) Cocking, T. T., *Pharm. Jour.*, 107 (1921), 226–227.

(6) Taxler, R. N., Baum, L. A. H., and Pitman, C. V., *Ind. Eng. Chem., Anal. Ed.*, 5 (1933), 165.

(7) Roller, P. S., *Ind. Eng. Chem.*, 22 (1930), 1206.

(8) Smith, G. F., Hardy, L. V., and Gard, E. L., *Ind. Eng. Chem., Anal. Ed.*, 1 (1929), 228.

Sir Ernest Rutherford (1871–1937) was was the recipient of the Nobel Prize for Chemistry in 1908 for his investigations of the decay of elements and the chemistry of radio-active substances.

A History of Blaud's Pills*

By M. L. Neuroth† and C. O. Lee‡

Pills of ferrous carbonate were introduced into medicine in 1831 in France. The inventor of the formula, so far as we know, was Dr. Blaud, head physician of the Hospital of Beaucaire, Gard. He was a corresponding member of the Acadamie Royale de Medicine and reported his product to the journal. It seems that the formula was well received and much praised.

In September (1831) Cottereau, reporting in the Bulletin Generale de Therapeutique, stated that a provincial medical man had some time previously communicated the composition of certain pills which he had found efficacious in cases of chlorosis to the Acadamie Royale de Medicine.

Cottereau went on to say that a pharmacist by the name of Guillard had made pills presumably similar to those of Blaud. The formula for them was as follows:

Subcarbonate of Potassium	ʒii
Crystals of Iron Sulfate well powdered	ʒii

These ingredients were made into 48 pills which were silver coated. Licorice, marshmallow or gum arabic were used as excipients.

Early in 1832 Blaud protested the report of Cottereau stating that the latter's formula was not identical with his own which was as follows:

℞	Sulfate of Iron, finely powdered	ʒi
	Carbonate of Potassium finely powdered	ʒi

These salts were made into a mass, using mucilage of tragacanth and powdered licorice as excipients, and divided into 96 pills.

It is evident that Blaud was the provincial medical man concerning whom Cottereau had made reference. In a letter to the Bulletin Generale de Therapeutique (2) Blaud later claimed that the composition of Pilules Antichlorotique was his invention. A translation of his letter from the French is as follows:

"It is to me that the composition of the antichlorotic pills belongs, (*i. e.*, the composition is my composition, my work, my formula) the formula of which M. Cottereau has given on page 290 of your excellent journal.

This formula does not at all conform to that which I have communicated to the Academy; the dose of the medicaments which compose it are there in quantity a half less than in mine. I hold that a remedy so precious, which has never failed in my hands and which I consider as a specific in chlorotic affections, may be used in suitable doses to obtain a complete success, I hasten to communicate to you the mode of preparation which I have always used.

℞	Sulfate of Iron, in fine powder, one ounce	
	Sub-carbonate of Potash, in fine powder, one ounce	

Mix intimately in a mortar and add tragacanth mucilage a sufficient quantity. Make a pilular mass by adding the proper amount of licorice powder, and divide into ninety-six pills. On triturating the mixture during about a half hour, there results a mass consistent enough to be divided into pills. Licorice powder is used only to keep the pills from adhering together.

The dose of the pills is not more perfectly indicated in the formula of M. Cottereau. One ought to take them as follows: the first three days one in the morning fasting, and a second in the evening on going to bed; the three following days one adds a third, which is taken in the afternoon; the seventh, eighth, and ninth days one takes two morning and evening; the tenth, eleventh, and twelfth days, two in the morning, two afternoon, and two in the evening; the three following days, three morning and evening; and thereafter nine per day until a perfect cure. I do not believe it necessary to take an infusion of buds of northern spruce or any other auxiliary medicament." Blaud.

The truth about Blaud's Pills is discussed editorially in the *Pharmaceutical Journal* (3) of 1906. The editor stated that "some marvelously inaccurate information" had been published about the original formula for Blaud's Pills in a German Medical paper. It does not appear, however, that there was any question as to Blaud's priority concerning the formula. There were, it appears, discrepancies as to the formula and the time of publication.

Boudet (4) in 1841 reported Simonin's modified procedure for the manufacture of

* An abstract of a thesis presented to the faculty of Purdue University in partial fulfilment of the requirements of the degree of Master of Science.

Presented before the Historical Section of the A. PH. A., Richmond meeting, 1940.

† Instructor in Pharmacy, Ohio Northern University, Ada, Ohio.

‡ Professor of Pharmacy, Purdue University, Lafayette, Indiana.

Blaud's Pills. This involved the use of honey which apparently produced a mass of a dark green color and good consistency. It was recognized at this time that saccharine substances prevented the oxidation of iron. No further modification of Blaud's Pills formula was made until the Codex Medicamentarius of 1866 appeared (5).

VALLET'S MASS

In connection with Blaud's Pills it might be well to suggest that the original formula for Vallet's Mass was reported to the Royal Academy of Medicine in 1837 and published in the *Journal de Pharmacie* in 1838 (5). It was contended that the mass was less apt to change with age than Blaud's Pills. Blaud took exception to this suggestion.

The original formula for Vallet's pill mass was as follows:

Sulfate of iron crystals, recently prepared according to the method of Bousdorff	500 Gm.
Carbonate of soda, pure	588 Gm.
White honey, very pure	306 Gm.
Syrup	*q. s.*

This formula somewhat modified was made official in the Codex of 1866 and through its subsequent revisions under varying titles. The formula is official in the 6th edition (1937) of the Codex Medicamentarius Gallicus as Pilules de Carbonate Ferreux. The synonym is Pilules Dites de Vallet. Inasmuch as the latest issue of the Codex has retained the Vallet's pill mass formula and omitted the Blaud's Pills formula one is led to conclude that perhaps there was something to the argument set forth more than a hundred years ago that Vallet's mass was more stable than Blaud's Pills. However, the Blaud's Pills formula is still official in the following pharmacopœias recently revised.

Deutsches Arzneibuch VI (1926)
British Pharmacopœia (1932)
Pharmacopœia Danica VIII (1933)
U. S. Pharmacopœia XI (1936)

This fact would indicate some differences of opinion concerning these two ferrous carbonate formulas.

COMPOUND PILLS OF IRON IN THE UNITED STATES PHARMACOPŒIA

Compound Pills of Iron appeared in the U. S. Pharmacepœia of 1830, Philadelphia edition. The formula was as follows:

Take of Myrrh, in powder, two drachms;
Carbonate of Soda
Sulphate of Iron, each, a drachm;
Syrup, a sufficient quantity.

The ingredients were formed into a mass and divided into 80 pills. It seems reasonable that ferrous carbonate would have been formed.

A formula of like nature also appeared in the U. S. Pharmacopœia of 1830, New York edition. It bore the title Compound Pills of Iron, synonym Griffith's Pills. The only difference between this formula and the one given above was that sugar replaced the syrup.

The U. S. Pharmacopœia II (1840 retained this formula making use of syrup instead of sugar as a massing agent. It was official in the U. S. Pharmacopœias III (1850), IV (1860), V (1870) and VI (1880) unchanged except that variations in the amounts of the constituents were made from time to time. No changes in the ratios of each were made. The synonym, Griffith's Pills, was not retained after 1840.

PILLS OF FERROUS CARBONATE

Pills of Carbonate of Iron were recorded in the U. S. Pharmacopœia II (1840). The formula was as follows:

Take of Sulfate of Iron, four ounces;
Carbonate of Soda, five ounces;
Clarified Honey, two ounces and a half;
Syrup
Boiling water, each a sufficient quantity.

Directions were given for preparing a mass of pilular consistence, but nothing was said about the number of pills to be made.

The U. S. Pharmacopœia III (1850) retained the formula increasing the amounts of the ingredients to twice those of the above formula. The U. S. Pharmacopœia IV (1860) varied the proportions of the constituents considerably from the original formula. This same formula appeared in the U. S. Pharmacopœia V (1870) without

change, but was not retained in the U. S. Pharmacopœia VI (1880).

BLAUD'S PILLS OFFICIAL IN THE U. S. PHARMACOPŒIA

The U. S. Pharmacopœia VII (1890) introduced Pills of Ferrous Carbonate similar to Blaud's Pills formula. Other synonyms were Ferruginous Pills and Chalybeate Pills. The formula was as follows:

Ferrous Sulphate, in clear crystals	16 Gm.
Potassium Carbonate	8 Gm.
Sugar	4 Gm.
Tragacanth in fine powder	1 Gm.
Althaea in No. 60 powder	1 Gm.
Glycerin	
Water, each a sufficient quantity to make a hundred pills.	

The details for making these pills was given with the statement that they should be freshly prepared when wanted.

This same formula was official in the U. S. Pharmacopœia VIII (1900), but the synonyms were not retained. However, the U. S. Pharmacopœia IX (1910) retained the previous formula and its three earlier synonyms. The substitution of monohydrated sodium carbonate for the potassium carbonate was ordered in the formula. This was a return, in part at least, to the Vallet's pill mass idea which has been discussed earlier in this paper. An assay procedure was introduced for the first time.

No significant changes appeared in this formula in the U. S. Pharmacopœias X (1920) and XI (1930). However, there is a note in the latter which permits these pills to be made by other methods if desired.

It is interesting that the U. S. Pharmacopœia VI (1880) was without a ferrous carbonate formula either of the Blaud's Pills or the Vallet's pill mass type. It is likewise of interest to note that prior to 1890 the ferrous carbonate formulas which were official in the U. S. Pharmacopœias were of the Vallet's pill mass kind and after that date they were all of the Blaud's Pills type.

UNOFFICIAL FERROUS CARBONATE FORMULAS

It seems that pills of ferrous carbonate have been a popular medicine for about a hundred years, but the ideal formula for the product has not been proposed as yet. The fact that ferrous carbonate masses are usually unstable has doubtless stimulated many workers to study the problem. As a consequence the literature is replete with formulas essentially alike, varying only in slight details.

In 1871 Maisch (6) presented a Blaud type of formula. He suggested that the hygroscopic effect of the potassium salt could be remedied by substituting sodium bicarbonate for it. Chipman (7) two years later discussed the use of honey and sugar in retarding the oxidation of iron in Blaud's Pills. In 1879 Pitschke (8) published a formula which, he claimed, would remain soft and green for a long time. It contained magnesium carbonate, glycerin and grape sugar as excipients. Another writer stated that a metallic luster could be given to pills by rolling them upon a hard board in coarsely powdered graphite moistened with a little alcohol.

Deupser (9) proposed the use of sodium borate in a formula. He claimed that it remained green for a long time and did not harden rapidly. Thompson (10) in 1887 proposed castor oil as one of the excipients.

During these years when our American pharmacists were suggesting many formulas for pills of ferrous carbonate, British pharmacists were also busy. Among those who contributed discussions upon the subject in the *Pharmaceutical Journal* were, Ince, Martindale, and others. Maben devised a formula for Pilula Ferri which was published in the British College of Physicians Formulary. It was made official in the 1890 additions to the British Pharmacopœia of 1885. Blaud's Pills was the synonym.

In 1899 Miehle (11) suggested a formula for pills containing equal parts of potassium carbonate and sodium bicarbonate. Dunning (12) later published a formula which contained glucose as one of the chief excipients. He maintained that this mass remained plastic for weeks.

After experimenting with various formulas for Blaud's Pills, Lowry (13) concluded that the bicarbonates are better than the carbonates and that sodium is more satisfactory than potassium for a good mass. He proposed a formula containing sodium

bicarbonate. Heat was used in the procedure and ether was one of the excipients.

In 1930 Lang (14) made use of exsiccated sodium carbonate in a formula and used dextrin and kaolin, glycerin, and water as the excipients. He claimed that his mass did not oxidize or harden with age. In spite of such a claim as this Witts (15) says that the ideal preparation of iron still awaits discovery.

From a résumé of the subject one becomes convinced that many hours of labor have been expended by a long list of workers in an effort to perfect an ideal mass of ferrous carbonate. The problem is still an intriguing one.

It is of interest to note that the formulas for Blaud's Pills in the pharmacopœias of the United States, Great Britain, France and Germany have not been altered greatly since 1890. Not much has been written about the problem since 1900. However, it seems that there is a revival of interest in it at the present. The results of our recent work and observations will appear in another paper.

REFERENCES

(1) Ince, J., *Pharm. J.*, 54 (1894), 131.
(2) Blaud, P. B., *Bull. Gen. Therap.*, (April 1832), 154.
(3) Anon., *Pharm. J.*, 77 (1906), 369.
(4) Boudet, M. F., *J. Pharmacie* (March 1841), through *Pharm. J.*, 1 (1841–1842), 70.
(5) Humphrey, J., *Pharm. J.*, 70 (1903), 643.
(6) Maisch, J. M., *Am. J. Pharm.*, 43 (1871), 307.
(7) Chipman, E. D., PROCEEDINGS, A. PH. A., 21 (1873), 600.
(8) Pitschke, I., *Pharm. Ztg.*, (1879), 750, through *Am. J. Pharm.*, 52 (1880), 87.
(9) Deupser, C., *Rundschau*, through *Am. Drug*,. 13 (1884), 228.
(10) Thompson, T., *Pharm. J.*, 46 (1887), 864.
(11) Miehle, F., *Apoth. Ztg.*, (Dec. 9, 1899), 741, through PROCEEDINGS A. PH. A., 48 (1899), 498.
(12) Dunning, H. A. B., *Drug Circ.*, 47 (1903), 226.
(13) Lowry, W. J., *Proceedings Md. Pharm. Assoc.* (1903), 89, through PROCEEDINGS A. PH. A., 52 (1903), 553.
(14) Lang, J. B., *Pharm. Acta Helv.*, 5 (1930), 53, through *Pharm. J.*, 125 (1930), 431.
(15) Witts, L. J., *Lancet*, I (1936), 1–5

"If you have knowledge, let others light their candles on it"—Margaret Fuller

Book Reviews

"Cushny's Pharmacology and Therapeutics," by EDMUNDS and GUNN. 852 pages. Lea & Febiger, 1940. Price, $6.50.

When a book has passed through twelve editions and has enjoyed the wide acceptance of this test in both medical and pharmacy schools for forty-one years, little can be said in comment on it. The changes in the present edition over the previous one are mainly in bringing the subject matter up-to-date, and in the inclusion of newer remedies. Among these is sulfanilamide and related compounds where an excellent job of correlation has been done in a difficult field. The temptation to introduce into this section material with unproved but fascinating implications must have been great. This has been avoided and consequently the student is properly prepared for future more controversial aspects of this field. The outstanding characteristic of this edition, as well as those which have immediately preceded it, seems to be a careful and intentional attempt to make this text useful to the student. There is probably no subject in the medical-pharmaceutical curriculum which lends itself to organization with such difficulty. Drugs often have many points of action and are used for so many different therapeutic purposes that the author of a textbook is confronted with the difficult task of classification and presentation in a logical form. The original author and his revisers have given evidence of awareness of this problem and have consciously set out to solve it in so far as our present knowledge of pharmacology will permit. To this end they have devised a system which is designed for "convenience in teaching the subject and ease of learning it." They have admirably succeeded. Some may object to occasional omissions of obscure, but pharmacologically interesting substances. Others might object to the use of so few references. But if one remembers the purpose for which the book was designed, it can be seen that these are advantages. For students, particularly those in pharmacy, proper orientation and sound fundamental organization are more important objectives than heterogeneous miscellanea, no matter how extensive. These objectives are admirably fulfilled by this text.
—J. M. DILLE

Vitamin E—A Symposium, under the auspices of the Food Group (Nutritional Panel) of the Society of Chemical Industry on April 22, 1939, at the School of Hygiene and Tropical Medicine, London, W.C.I., England. Monograph of 88 pages, bound. Chemical Publishing Co., Inc., New York. Price $2.00.

The monograph, which is edited by A. L. Bacharach of the Glaxco Laboratories, Middlesex, and Professor J. C. Drummond of the Biochemistry De-

partment, University College, Gower Street, London, is divided into three parts. Part I deals with the chemical structure and properties of tocopherol (vitamin E) under six divisions, or heads; the chemistry of vitamin E by Professor A. E. Todd of the University of Manchester; synthetic analogs and homologs of vitamin E; and the stability of tocopherol and tocopherol esters by research workers on the subject. Part II is devoted to a consideration of the physiological action of vitamin E and the consequences of vitamin E deficiency. Part III comprises reports of work done on the treatment of habitual abortion in the Maternity Hospital, Leeds, and Jessop Hospital for Women, Sheffield, and the University of Sheffield.—E. G. E.

An Introduction to Materia Medica and Pharmacology, by HUGH ALISTER MCGUIGAN and ROBERT ALISTER MCGUIGAN with ELSIE E. KRUG. 871 pages. St. Louis: The C. V. Mosby Company, 1940. Price, $3.50.

The second edition of this interesting text is in reality a handbook of materia medica and pharmacology designed primarily for instruction of nurses. Unlike the senior author's "Applied Pharmacology," this book is conveniently separated into chapters and offers as satisfactory and rational a classification of materia medica and pharmacological subjects as is possible at the present time. Much space, of course, is devoted to the purely pharmaceutical information which nurses are required to know, and official and semi-official drugs in elementary chemistry are listed very concisely. Beautifully colored plates of important drug plants, as well as of certain pharmaceutical preparations, constitute one of the best features of the book. The very satisfactory handling of the pharmacological section of the volume enables it to meet the requirements of the nursing profession and makes it profitable reading even for the medical student. An interesting feature of the text are the questions with which the various chapters conclude. The book will be welcomed by those reviewing the subject with an eye to the state examinations. The excellent format of this work also serves to make it one of the best of that type which has come to the attention of the reviewer. —D. I. M.

The Physiology and Pharmacology of the Pituitary Body. Volume II, by H. B. VAN DYKE, M.D. xiv + 402 pages. University of Chicago Press. Price, $4.50.

This monograph of some 400 pages is not, as might be supposed from its title, a second volume of a work on the pituitary gland but really a supplement to Volume I, which contains a critical digest by the same author of experimental and clinical literature appearing since 1935 on the master gland of the human body. In other words, it covers the scientific output on the subject during the period extending from 1935 to the early part of 1938. As books go it is therefore already out of date in this year of 1940. The prodigious amount of work being done on the pituitary can be gathered from the fact that approximately 750 articles dealing with that gland are published yearly and that the bibliography of the volume before us contains 1418 titles. A perusal of the pages reveals the complexity of the subject and the remarkably ingenious methods which have been employed in investigation of the pituitary gland. The volume begins with a chapter of thirty-two pages on its anatomy, followed by lengthy sections devoted to the various functions of the anterior pituitary lobe, and it is only in the ninth chapter that the writer discusses the important new discoveries made regarding the chemistry and pharmacology of the posterior lobe of *Pars neuralis*. A full chapter is delegated to the subject of regulation of growth by the pituitary. Two other chapters deal with the gonadotropic hormones of the pituitary and the relation of these hormones to pregnancy, and to certain neoplasms. A separate section is devoted to the *Pars glandularis* in relation to the development of the mammary glands and lactation. Another section of 24 pages discusses the thyrotropic function of the pituitary. A very important chapter describes the interrelationship between *Pars glandularis* and the adrenal glands, and its influence on metabolism of carbohydrates, lipoids, proteins, minerals and function of parathyroid glands. A separate chapter is given to the regulatory function of the pituitary in connection with chromatophores. The portion dealing with the posterior lobe presents the latest knowledge concerning chemistry and pharmacology of that still not completely understood part of the pituitary gland. An excellent discussion at the end deals with *Pars neuralis* as a gland of internal secretion. This monograph is richly illustrated with diagrams, some of them in colors. An appendix presents the structural formulas of the principal hormones of natural origin. The whole work is a scholarly digest of an intricate subject by one who has gleaned his information in a considerable degree from original research and is thorougly conversant with literature on the subject. The statement on the title-page that Dr. Van Dyke was formerly professor of pharmacology at the University of Chicago and the Peiping Medical College and is now director of pharmacology at the Squibb Institute should not be overlooked. There is a moral in this bit of information. It points to the significant fact that the number of distinguished pharmacologists associated with privately endowed industrial laboratories is increasing while the roster of those connected with pharmacological departments of the universities dwindles in proportion.—D. I. M.